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FLUORIDE DRINKING WATERS

National Institute of Dental Research
Bethesda, Maryland

1962

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FLUORIDE DRINKING WATERS

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service

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FLUORIDE
DRINKING WATERS

A selection of Public Health Service papers on dental fluorosis and dental caries; physiological effects, analysis and chemistry of fluoride

F. J. McClure, *Editor*

Details of illustrations in
this document may be better
studied on microfiche.

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institute of Dental Research, Bethesda, Maryland

I

The first article of this volume was published in 1901; the majority were published since 1929; the most recent will appear in a scientific journal in 1962. Each article is reprinted with the permission of the journal in which it is published. Research pertinent to fluoride is continuing at the National Institute of Dental Research and in other areas of the Public Health Service and will provide additional publications.

Public Health Service Publication No. 825
- 1962 -

II

PREFACE

"Sir, I have the honor to submit the following report: On the examination of certain Italian emigrants embarking at this port, one is struck with the frequency of a dental peculiarity common among the inhabitants of the littoral and known as "denti di Chiaie" or "Chiaie teeth." This defect was first described by Prof. Stefano Chiaie, a celebrated Neapolitan, and bears his name."

Thus, Past Assistant Surgeon, U.S. Marine Hospital Service, J. M. Eager, began an official communication to the Surgeon General of the Public Health Service dated October 7, 1901, Naples, Italy. Consulting Specialist in Child Hygiene, U.S. Public Health Service, Dr. Fredrick S. McKay, collaborating with Dr. G. V. Black in 1916 and with Surgeon Grover A. Kempf in 1931, again described this dental lesion occurring in the United States and known by that time as mottled enamel. Dr. McKay and Dr. Kempf emphasized the endemic character of mottled teeth and thought that the etiologic factor was "definitely associated with the water supply." Soon thereafter, Dr. H. Trendley Dean observed variability in the severity and incidence of mottled teeth, reflecting as Dr. Dean surmised, quantitative variations in a causative factor present in the water supply.

In 1931, as reported independently in three publications, endemic mottled enamel became associated with drinking waters containing remarkably small quantities of fluoride, only slightly above one part in one million parts of water.

This conclusive evidence that fluoride in the drinking water was the etiologic factor of endemic mottled enamel was to be followed by two equally impressive and classic discoveries. First was the evidence that although mottled, the teeth produced by fluoride-bearing drinking waters had acquired a remarkable immunity to dental caries. Secondly this particular immunity was produced by as little as 1.00 ppm of fluoride in drinking water and this low quantity of fluoride did not cause mottled enamel.

In due course of time the pronounced cariostatic effect of the direct addition of fluoride to public water supplies, known as water fluoridation, was conclusively documented. Impressive evidence of the safety of drinking water containing 1.00 ppm of fluoride was evident in the satisfactory health status of large population groups exposed to drinking waters containing, by nature, low levels of fluoride. Research at the laboratory level added further knowledge on the physiological and dental effects of fluoride.

Water fluoridation for the control of dental caries thus has become an acceptable public health procedure conclusively documented as to its benefits to the dental health of humanity. Within the brief span of little more than half a century, investigators cooperating in a number of research disciplines accomplished this momentous benefit to dental health. A fitting reward for their efforts is the official endorsement by the world's scientific, dental, medical and public health organizations, of water fluoridation as a practical, safe and remarkably effective means for the partial control of dental caries.

It is with much satisfaction that I present this compilation of the fluoride publications of the Public Health Service. I congratulate the fluoride and dental health investigators in the Public Health Service as well as those in other disciplines and organizations in the United States and other parts of the world.

Francis A. Arnold, Jr.,
Director National Institute
of Dental Research,
U.S.P.H.S., 1962.

CONTENTS

Page

ENDEMIC DENTAL FLUOROSIS (Mottled Enamel) 1

RELATION OF DENTAL CARIES TO FLUORIDE DRINKING WATERS 83

FLUORIDATION OF DRINKING WATER AND CONTROL OF DENTAL CARIES:
- Reduction in dental caries by fluoridated drinking water 197
- Engineering problems in water fluoridation , ... 257
- Caries inhibition by fluoride not provided by community drinking water . . . 282

TOPICALLY APPLIED FLUORIDE AND DENTAL CARIES CONTROL 305

THE PHYSIOLOGICAL EFFECTS OF FLUORIDE—The health status of human populations exposed to fluoride drinking water 343

EXPERIMENTAL LABORATORY RESEARCH RELATIVE TO EFFECTS OF FLUORIDE:
- Experimental dental fluorosis 461
- Inhibition of experimental dental caries by fluoride 470
- Fluoride metabolism and retention in calcified tissues 495

QUANTITIVE ANALYSIS AND CHEMICAL REACTIONS OF FLUORIDE:
- Analysis of fluoride 561
- Reactions of fluoride ion with enamel and dentin 603
- Water defluoridation 618

AUTHOR INDEX 635

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The Dental Research Building of the National Institute of Dental Research was dedicated on May 26, 1961, with ceremonies presided over by Dr. Francis A. Arnold, Jr., Director of the Institute. Dr. Emory W. Morris, President of the W. A. Kellog Foundation, gave the principal address. Official dedication of the Building was performed by the Secretary of the Dept. of Health, Education, and Welfare, the Honorable Abraham A. Ribicoff. Built at a cost of four million dollars, the structure's five floors provide over 42,000 square feet of working space, for 200 scientific and technical personnel engaged in research on oral and related diseases. Completion of the building marked the culmination of a decade of effort, by many interested groups, particularly the American Dental Association.

Seated from left to right in the photograph are Dr. Frank J. McClure, Dr. Francis A. Arnold, Jr., Dr. H. Trendley Dean, and Dr. Elias Elvove.

In June of 1948 the National Institute of Dental Research was established by act of the United States Congress and Dr. H. Trendley Dean became its first Director. Dr. Dean occupied this position until his retirement in 1953 at which time he was succeeded by Dr. Arnold, the present Director. Dr. Elias Elvove served as Chemist with the Public Health Service from 1907 until he retired in 1953. Together with Dr. McClure these men constituted the initial group of investigators in the Public Health Service studying the relation of fluoride to dental health, its analysis and its physiological effects. Dr. Dean was commissioned in the Dental Corps of the Public Health Service in 1921; Dr. Arnold was commissioned in 1936. Dr. McClure, now Chief, of the Laboratory of Biochemistry of the National Institute of Dental Research, began his fluoride research in the Public Health Service in 1936.

ENDEMIC DENTAL FLUOROSIS (Mottled Enamel)

Mottled enamel is an endemic hypoplasia of the permanent teeth, produced by the ingestion of toxic quantities of fluoride in drinking water. Its macroscopic pathology is classified according to severity as "questionable," "very mild," "mild," "moderate," "moderately severe," and "severe." Depending on this classification, the mottled enamel index of a community may be one of the following: "negative," "borderline," "slight," "medium," "rather marked," "marked" and "very marked." There is a direct quantitative relation between the fluoride content of drinking water and clinical manifestations of dental fluorosis. In amounts not exceeding 1.00 ppm in the drinking water, fluoride causes no significant development of mottled enamel. Where climatological factors markedly affect the intake of drinking water, the severity of dental fluorosis may be influenced by a variable ingestion of fluoride.

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DENTI DI CHIAIE TEETH (Chiaie Teeth)

By J. M. Eager

Naples, Italy,
October 7, 1901

Sir: I have the honor to submit the following report: On the examination of certain Italian emigrants embarking at this port, one is struck with the frequency of a dental peculiarity common among the inhabitants of the Italian littoral and known as "denti di Chiaie," or Chiaie teeth. This defect was first described by Prof. Stefano Chiaie, a celebrated Neapolitan, and bears his name.

The impairment of the teeth, often not amounting to more than a mere imperfection, is of particular interest, owing to the fact that Italians who are subjects of the defect frequently present themselves before the medical practitioner in the United States. The deterioration, however, is an acquired one, due to local geological conditions and so, because of altered hygienic surroundings, will not pass beyond the present generation of Italians in America. Strong well-formed teeth not particularly prone to decay appear to be the rule among young Italians when they have not been subjected to the influence during infancy of the causes of Chiaie's disease. The etiology seems to be connected with volcanic fumes or the emanations of subterranean fires, either fouling the atmosphere or forming a solution in drinking water. In Naples it is more often attributable to water than to the air, and since the Serino water, brought in conduits from a distant mountain height, has been in use and local wells condemned the incidence of the disease among infants has greatly diminished. Formerly nearly all children living in the section known as Santa Lucia, along the Riviera, and at Posillipo were affected.

The people of Pozzuoli, a town of 16,000 inhabitants, situated 5 miles from Naples, are marked off from the people of neighboring places by their distinguishing characteristic of black teeth (denti neri), apparently strong and serviceable, but devoid of enamel and hideously dark. The environs of Pozzuoli are everywhere volcanic. Close at hand is the Solfatara, a half-extinct crater full of cracks from which gases are constantly issuing. Some of the inhabitants of Pozzuoli drink the water of springs, a water necessarily charged under pressure with volcanic fumes; all of them are constantly living in an atmosphere filled with noisome gases.

The theory most generally received in Italy is that these gases have a selectively hurtful effect on enamel formation in early childhood, but that the growth of the other dental tissues is not interfered with. When the cause is active during the entire period of second dentition, the whole tooth is bereft of enamel and becomes perfectly black. If the growing teeth are exposed for but a short time at the commencement of their formation, only the cutting edges of the upper incisors may be affected and the appearance, when the teeth are matured, is as if they had been browned by tobacco smoke in the same way that a meerschaum pipe is colored by smoking. Sometimes the teeth have the repulsive look of fever patients' teeth when smeared with sordes, except where covered by the half-parted lips.

Among the better class of Italians living inland, it is the custom to go to the seashore in summer. Naples has always been a popular resort, and as a result of the temporary exposure of children brought with their parents to Naples at the time when Serino water was not used, it is frequent to see among well-to-do people an otherwise handsome face marred by a line of fine, black markings crossing the incisor teeth in a horizontal direction. This fault of development is known among Neapolitans as "denti scritti" or writing on the teeth. The marking, when present on finely formed, white teeth, resembles the diminutive lettering which is sometimes done on seashells for purposes of ornamentation.

The different forms of detioration which are grouped under the name of "denti di Chiaie" are thus seen to be quite unlike any other dental disease and not at all likely to be confounded with Hutchinson's teeth, mercurial teeth, caries, or other maladies of the teeth.

MOTTLED ENAMEL IN A SEGREGATED POPULATION

By Grover A. Kempf and Frederick S. McKay

Mottled enamel has an intersting history. An article on this condition by Passed Asst. Surg. J. M. Eager, of the United States Public Health Service, then stationed in Naples, Italy, appeared in the Public Health Report: in 1901. Dr. Eager noticed this condition of the enamel among Italian emigrants, particularly those coming from Pozzuoli, a community about 5 miles from Naples. This dental defect was called "denti di Chiaie," after Prof. Stefano Chiaie, who first described the condition. From Doctor Eager's description it is recognized as the same enamel defect found in various places in the United States and in other countries, and now known as "mottled enamel."

Incidentally, and curiously enough in the light of what is to follow later in this report, it was reliably reported to one of us (McKay) during an investigation of this Italian district in 1927, that Pozzuoli had changed its water supply and was no longer afflicted with this condition.

It was not until 1916 that further information appeared in the literature, when Dr. G. V. Black and Dr. Frederick S. McKay published a series of joint articles in *Dental Cosmos* on this condition. These men were the first in the United States to make an actual study of this enamel defect and to publish their results.

The term "mottled enamel" was first used by Black as a descriptive name for the condition. His description is quoted as follows:

> The most essential injury occurring in this mottled enamel is the appearance of the teeth. * * * The teeth are of normal form, but not of normal color. When not stained brown or yellow, they are a ghastly, opaque white that comes prominently into notice whenever the lips are opened. * * * In many cases the teeth appear absolutely black. Mottled enamel is distinguished especially by the absence

> of cementing substance between the enamel rods in the outer fourth, more or less, of the enamel, and presenting great variety of color, rendering it totally different from anything else I have known.

The outer glazed enamel surface, or Nasmyth's membrane, is present and appears to be normal, except in what have been termed the "corroded" cases. The associated pigmented or stained phase of mottled enamel is called "brown stain." This brown stain does not occur in all cases of mottled enamel, and it seems to be limited to the labial surfaces of the upper incisors and cuspids. Chemical analysis of the "brownin," or pigment, has been made, and it is said to be manganese.

It is extremely rare to find mottled enamel on any of the temporary teeth. A very few cases have been observed in the various affected districts in which there was a slight flecking of the typical white spots on the enamel of the temporary molars, but never to our knowledge has the brown stain been observed on any temporary teeth.

This typical brown stain can not occur unless the enamel bears the fundamental mottled defect; but not all teeth with mottled enamel are stained. McKay is of the opinion that the brown stain appears after the mottled teeth have erupted.

Noyes, in Dental Histology and Embryology, expresses the hypothesis that "the enamel rods and the cementing substance have a different origin, or are formed by different cells, and that pathological conditions may prevent the formation of one and not of the other."

In Black's first description of this lesion, it was made to appear that the enamel rods were themselves intact and of normal form and contour, but with the cementing substance absent. It has since been made evident, however, through the work of J. Leon Williams, that in some cases at least the rods themselves have been diminished in contour.

McKay has investigated the condition in several affected districts in Colorado, Texas, Virginia, and Arizona. His results were published in *Dental Cosmos*, May to August, 1916. In 1917, he studied several endemic areas in South Dakota, and later extended his investigations to include areas in Idaho and California. Recently other centers have been reported in Illinois, North Dakota, and Minnesota.

The condition occurs in various parts of the world, notably in Italy, certain of the Bahama Islands, Barbados, Holland, Cape Verde Islands, China, Mexico, Spain, Argentina, and other South American countries and in South Africa. Wherever it occurs the characteristics of the enamel defect are the same. Recently McKay noticed this condition in the photograph of a sheik of the desert published in the National Geographic Magazine.

The nature of the defect can readily be seen in the photographs of several typical cases presented herewith.

The disfiguring effect of mottled enamel can readily be appreciated. This condition is not subject to improvement or alteration through natural means, but only through a difficult operative procedure. When we also realize that every child who is born and reared in an endemic area is practically certain to have this disfigurement in some degree, we can readliy understand that this is a problem worthy of solution.

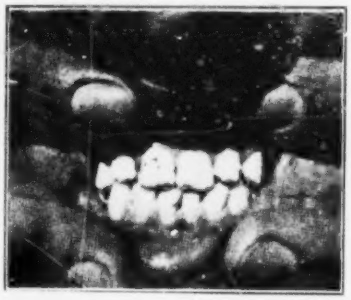
The time of development of the enamel of the deciduous and permanent teeth is of great importance in considering the etiology of mottled enamel, and the accompanying figures are taken from Burchard and Inglis, who used Peirle's table of calcification.

The ages are subject to some variation, and the mottling of the enamel in itself serves as an indication of the age when the enamel of the permanent teeth was developed in the child.

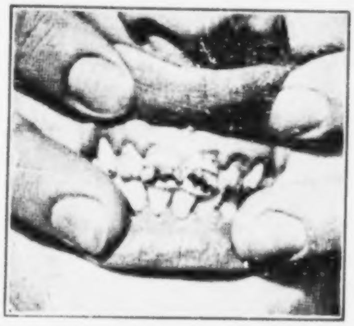
As previously stated it has been held that the temporary teeth are free from this defect, but in the survey with which this report deals, some slight indications of mottling of the temporary teeth were noted. In explanation of this it may be stated that the formation of the enamel of these teeth has practically been completed at the time of birth or very soon afterward, but this must undoubtedly be subject to fairly wide variation. Some children might not have all enamel of the temporary teeth developed until several months or even a year after birth. After the protective influence of the placental circulation has been terminated, the enamel remaining to be developed is affected by the etiological factors in the endemic area. This could be particularly true in the premature baby. It follows, therefore, that there may be no absolute reason against mottling of some of the temporary teeth.

The following table from Burchard and Inglis (Dental Pathology and Therapeutics, seventh edition. Lea & Febiger, Philadelphia and New York, 1926) gives the approximate ages for the eruption of the permanent teeth:

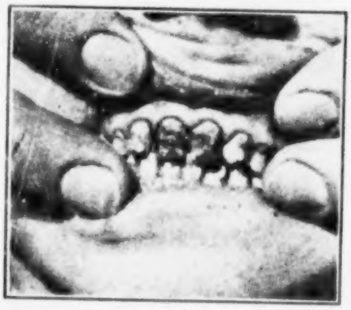
First molars	5½ to 7 years.
Central incisors	7 to 8 years.
Lateral incisors	8 to 9 years.
First bicuspids	10 to 11 years.
Second bicuspids	11 to 12 years.
Cuspids	12 to 14 years.
Second molars	12 to 15 years.
Third molars	16 to 20 years or more.



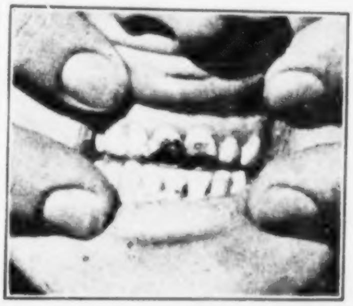
A case of mottled enamel without stain. Note paper-white appearance of the enamel.



Case of typical mottled enamel with brown stain. The teeth are well formed and the stain is limited to the labial surface of the upper incisors.



Extreme type with corrodedlike condition of the enamel.



Stained and corroded teeth.

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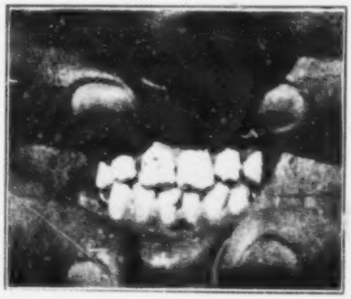
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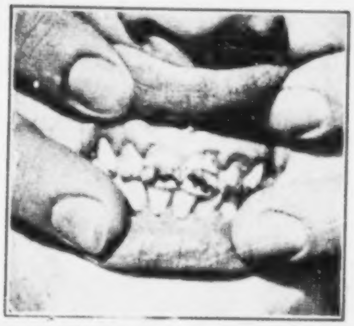
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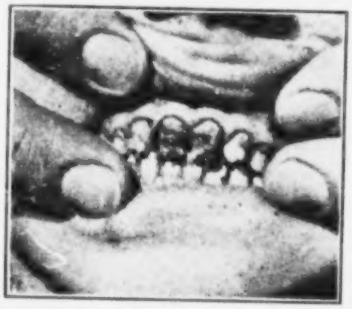
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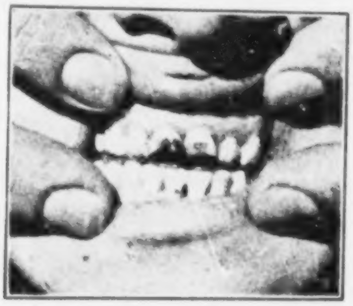

Stained and corroded teeth.

For convenience we will divide the permanent teeth into three groups, as did Black and McKay in their reports:

	Calcification		Eruption
First group:			
First molars	1 to 5 years		5½ to 7 years.
Incisors	1 to 7 years	central	7 to 8 years.
		lateral	8 to 9 years.
First bicuspids	7 to 8 years		10 to 11 years.
Second group:			
Second bicuspids	7 to 8 years		11 to 12 years.
Cuspids	7 to 8 years		12 to 14 years.
Second molars	5 to 9 years		12 to 15 years.
Third group:			
Third molars	9 to 12 years		16 to 20 years or more.

The endemic area with which this report deals was reported to the Public Health Service in 1927, and centers in the town of Bauxite, Ark., which was established in 1901 to provide homes and a social environment for the employees of a mining company.

The original supply of water for domestic purposes came from shallow surface wells and a few springs. As the population increased, a larger supply was required, and in 1909 a deep well of 255 feet depth was drilled, later augmented by two other wells close by. Water from these wells was piped into the homes.

Following this, most of the shallow wells were gradually filled, and within the community proper the deep wells were the chief source of the water supply. This deep well water has a disagreeable alkaline taste, and many of the families continued the use of spring water for drinking, especially during the warm months.

The evidence collected during the examination of the children in the town school, consisting of the elementary grades and the high school, can be summarized as follows:

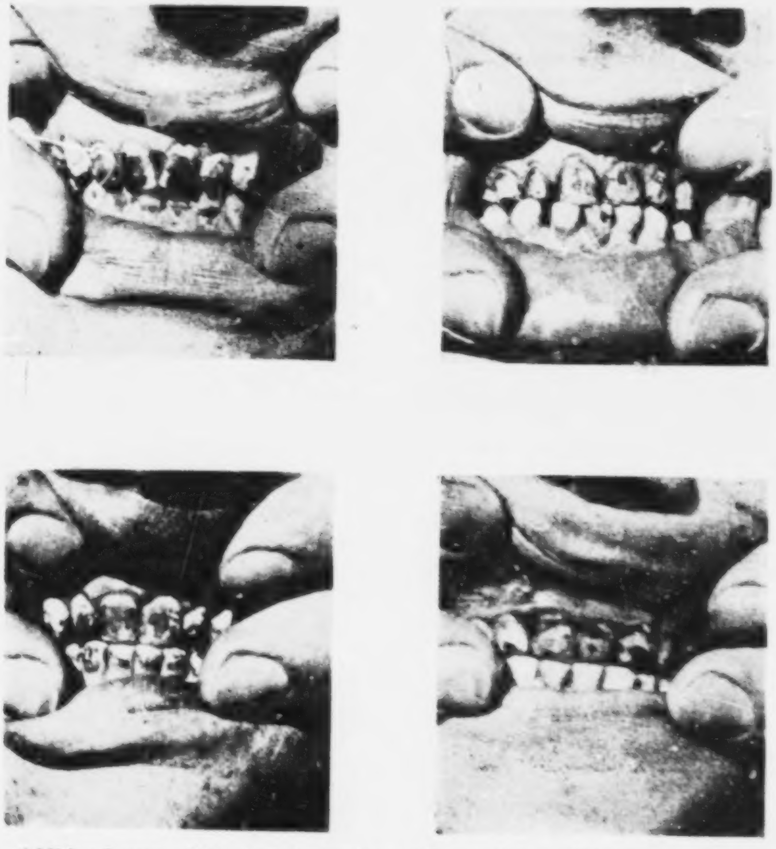
1. No cases of the enamel defect were found which antedated the introduction of the deep well water.
2. The oldest individual found with this enamel defect was born about the time that the deep well water was introduced.
3. All individuals in the community who had used the deep well water during any considerable period of enamel formation exhibted this defect
4. No individual in the community whose enamel had developed elsewhere exhibited the defect.
5. Certain individuals who, although residents of the community and attending school there, but who actually lived beyond the distribution of the deep well water and depended upon the original shallow wells, exhibited only normal enamel.

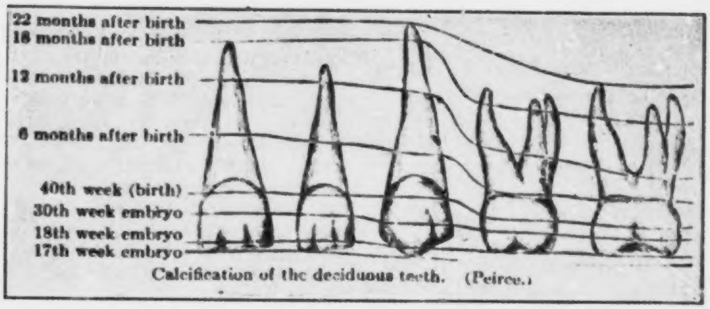
Evidence supporting these postulates is presented in the tables appearing later in this report. In no district so far observed by one of the authors (McKay) has the evidence pointed so directly to a relation between the use of a certain definite water and the production of this enamel defect.

It is necessary to consider the possibility, however, that some factor in the food supply of these individuals, either before or after birth bears some relation to the defect in question.

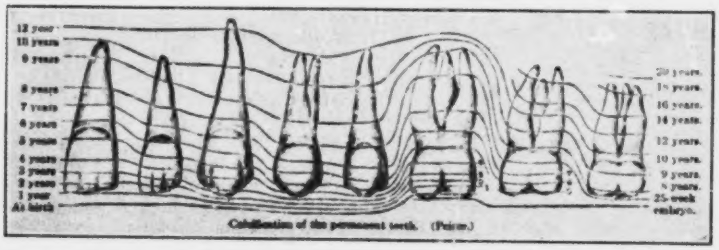
In considering this point it is important to note that the enamel of the temporary teeth is formed almost entirely before birth, and is, therefore, directly dependent upon the prenatal nutrition. These teeth, however, practically never show the defect. The permanent teeth do not commence to form until about one year after birth (according to the average), and, hence, can not be even remotely related to the maternal nutrition.

The source of the food supply of these people is from a central store. The staple articles here, under the complexities of present-day civilization, are produced and manufactured elsewhere. In season certain articles are procured from surrounding truck farmers. It may be said that these locally grown products are reflective of the chemistry of the local soil. But the water from the shallow


Additional cases which show the stained and corroded condition of the teeth.



Calcification of the deciduous teeth. (From Dental Pathology and Therapeutics, seventh edition, by Burchard and Inglis).



Calcification of the permanent teeth. (From Dental Pathology and Therapeutics, seventh edition, by Burchard and Inglis).

or surface wells is equally indicative of soil chemistry, and such wells are associated with normal enamel.

The diet of such of these persons exhibiting the enamel defect was in nowise different, except in the source of the water, from that of persons in the same environment who had escaped the defect.

In the children examined in the school there was ample opportunity to observe all gradations of tooth eruption, from those in whom the first permanent teeth were just appearing, to those with all permanent teeth erupted.

The children were requested to fill a questionnaire giving the place of birth and a history of places of residence since birth, with dates or ages at time of each change of residence. Each child was examined by McKay under natural illumination and without previous knowledge of the child's residence. The facts regarding age, time in Bauxite, etc., were verified by the child after the examination. This was later checked from the rental records of the company.

In no community previously examined by McKay were the facts relating to age and place and duration of residence so accurately recorded as in Bauxite, owing to access to the mining company's records and rent rolls, and the close cooperation given by the company's director of social relations among the employees.

A total of 458 children from 5 to 18 years of age were examined in the schools of Bauxite. Mottled enamel of some teeth were found in 202 cases, or 44 per cent. In evaluating this percentage it is necessary to recall again the immunity of temporary teeth and also to consider the imported individuals, by which we mean those persons who had grown the enamel on such permanent teeth as were erupted at the time of the examination elsewhere, before becoming residents of Bauxite. If some local condition at Bauxite were responsible for the defect, such enamel would of course be normal. The complete picture could be revealed only by being able to see the condition of the unerupted permanent teeth of these native children and similar teeth of the imported individuals upon which the enamel had been grown in Bauxite.

Table 1, presenting data on children who were born in Bauxite, gives the age of the individual at the time of examination, the number of children for each age, and the condition of the enamel of the first, second, and third groups of permanent teeth. There were 66 children in this group who were born in Bauxite. Of these, 63 had permanent teeth erupted and 61 had mottled enamel of the first group of permanent teeth. Three children had no permanent teeth erupted, and only two children had normal enamel of the permanent teeth.

One will note that the ages of the children of this group ranged from 5 to 18 years, and 43 of them had not erupted their second group of permanent teeth. The 23 children who had their second group of permanent teeth had mottled enamel of these teeth. The one child of 18 years, who had erupted the third molars, had mottled enamel of all his teeth.

There were two children with normal enamel of the first permanent group. One of these was 6 years of age, and his second group of permanent teeth had therefore not erupted; the other was 17 years of age and showed only slight mottling on his second group of permanent teeth. The 14-year-old sister of this second case had definite mottling of all her permanent teeth. These two families had access to spring water as well as to the central supply.

TABLE 1.—*Condition of the enamel in children who were born in Bauxite, Ark., and had lived there all their lives, using municipal deep-well water supply*

Age in years, at time of examination	Total number of children	First group				Second group			Third group		
		Mottled but not stained	Mottled and stained	Normal	Not erupted	Mottled	Normal	Not erupted	Mottled	Normal	Not erupted
5	1	1						1			1
6	10	7	1	[1] 1	1			10			10
7	15	11	2		2			15			15
8	10	7	3			2		8			10
9	8	4	4			2		6			8
10	4	2	2			1		3			4
11	2		2			2					2
12	2	1	1			2					2
13	7	2	5			7					7
14	3	1	2			3					3
15	1		1			1					1
16	1		1			1					1
17	1			[2] 1		1					1
18	1	1				1			1		
Total	66	37	24	2	3	23		43	1		65

[1] City water available in house but was not used for cooking or drinking.
[2] City water available in house but used spring water for drinking most of the time.

TABLE 2.—*Mottled enamel of the first group*[1] *of permanent teeth, among children in Bauxite, Ark.*

Ages between birth and 6 years during which child lived in Bauxite and used municipal deep well water supply	All ages	Age at time of examination																
		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Birth to 6 or over:																		
Total examined	77	10	12	11	11	5	3	3	8	5	2	3	3	1	----	----	----	----
Numbered mottled	75	9	12	11	11	5	3	3	8	5	2	3	2	1	----	----	----	----
Under 2 to 6 or over:																		
Total examined	16	1	4	2	3	1	4	1	----	----	----	----	----	----	----	----	----	----
Number mottled	16	1	4	2	3	1	4	1	----	----	----	----	----	----	----	----	----	----
2 to 6 or over:																		
Total examined	21	2	4	1	----	3	----	2	3	1	1	1	1	2	----	----	----	----
Number mottled	16	2	2	1	----	3	----	1	3	----	1	1	1	1	----	----	----	----
3 to 6 or over:																		
Total examined	24	3	4	3	2	2	2	----	2	----	4	2	----	----	----	----	----	----
Number mottled	12	1	1	2	----	2	2	----	----	----	2	2	----	----	----	----	----	----
4 to 6 or over:																		
Total examined	26	----	3	2	5	----	4	7	----	2	----	----	1	----	----	1	----	1
Number mottled	10	----	----	2	2	----	----	5	----	----	----	----	----	----	----	1	----	----
5 to 6 or over:																		
Total examined	22	3	2	3	1	1	1	1	3	1	3	3	----	----	----	----	----	----
Number mottled	2	----	----	----	----	1	----	1	----	----	----	----	----	----	----	----	----	----

[1] First group consists of first molars, incisors, and first bicuspids.

This table shows the condition of only the first group of permanent teeth, by age of the child at the time of examination, and the ages between birth and 6 years during which the children lived in Bauxite, and used the municipal deep well water supply.

There were a total of 77 children who lived in Bauxite from birth to 6 years or more of age. Of these, 75, or 97.4 percent, had mottled enamel of the first group of permanent teeth in some degree. Of 16 children who lived in Bauxite from under 2 years of age to 6 years or over, all had mottled enamel. Of 21 children who lived there from 2 years of age to 6 years or over, 16, or 76 percent had mottled enamel. Of 24 children who lived there from 3 years of age to 6 years or over, 12, or 50 percent, had mottled enamel. Of 26 children who lived there from 4 years of age to 6 years or over 10, or 38.5 percent, had mottled enamel. Of 22 children with residence in Bauxite from 5 years of age to 6 years or over, only 2 or 9 percent, had mottled enamel in some degree of the first group of permanent teeth. The enamel of these teeth is considered to be developed from sometime in the first year of life to about the sixth or seventh year. There is a definite relation between the time lived in Bauxite between birth and 6 years of age and the condition of the enamel. The fact that two children who arrived in Bauxite at 5 years of age had defective enamel indicates that the time of the development of enamel is subject to some variation. Of course there is the possibility that these children developed defective enamel before their arrival in Bauxite.

The definite decrease in the percentages, however, is striking, ranging from 100 percent down to 9 percent, and furnishes what seems to be the most reliable index to what is, after all, the remarkable uniformity in the rate of development of the teeth according to age.

Table 3 shows the condition of the enamel of the second group of permanent teeth with the age of the child at the time of examination and the ages between birth and 9 years or over during which he had lived in Bauxite.

A total of 28 children lived in Bauxite from birth to 9 years of age, and all of these children had mottled enamel of the second group of permanent teeth. Four children had lived there from under 2 years of age to 9 years or over, and all had mottled enamel of the second group. There were 10 children with Bauxite residence from 2 years of age to 9 years or over and all had mottled enamel of the second group.

The percentage of defective enamel of the second group with residence from 3 years of age, 4 years, 5 years, 6 years, 7 years, and 8 years of age to 9 years or over, was respectively as follows: 81 percent, 93 percent, 55 percent, 58 percent, 21 percent, and 12 percent.

The condition of the third group of permanent teeth is of great interest. This group consists only of the third molars, or "wisdom teeth." These teeth are supposed to calcify from about 9 years to 14 years of age.

According to the evidence bearing upon the influence of the environmental factors of the etiology of mottled enamel, the third molars of a child coming into an endemic district after the age of 8 or 9 would present the typical lesion and the remainder of the teeth (formed elsewhere) would be normal.

We were able to examine 14 people from 15 to 24 years of age who had lived in Bauxite two years or more during the time of calcification of the third molars.

Table 4 presents the age arrived in Bauxite, present age, number of years lived in Bauxite, and the condition of the enamel of the third molars. These 14 cases will be considered individually as the data appear of importance in etiological evidence.

The first of these persons came to Bauxite at 4. He lived there 18 years. The enamel of the first group is normal; that of the second and third groups is mottled. This is a typical case. The next was born in Bauxite and had lived there all his life. The enamel of all his teeth

TABLE 3.—*Mottled enamel of the second group*[1] *of permanent teeth among children in Bauxite, Ark.*

Ages between birth and 9 years during which child lived in Bauxite and used municipal deep-well water supply	All ages	Age at time of examination													
		9	10	11	12	13	14	15	16	17	18	19	20	21	22
Birth to 9 or over:															
Total examined	28	2	1	3	3	7	5	2	2	2	1	----	----	----	----
Number mottled	28	2	1	3	3	7	5	2	2	2	1	----	----	----	----
Under 2 to 9 or over:															
Total examined	4	----	----	3	1	----	----	----	----	----	----	----	----	----	----
Number mottled	4	----	----	3	1	----	----	----	----	----	----	----	----	----	----
2 to 9 or over:															
Total examined	10	----	1	----	2	3	1	----	1	1	1	----	----	----	----
Number mottled	10	----	1	----	2	3	1	----	1	1	1	----	----	----	----
3 to 9 or over:															
Total examined	11	----	1	2	----	2	----	4	2	----	----	----	----	----	----
Number mottled	9	----	1	2	----	1	----	3	2	----	----	----	----	----	----
4 to 9 or over:															
Total examined	15	3	----	2	3	----	4	----	----	1	----	----	1	----	1
Number mottled	14	2	----	2	3	----	4	----	----	1	----	----	1	----	1
5 to 9 or over:															
Total examined	9	----	----	1	----	3	1	3	1	----	----	----	----	----	----
Number mottled	5	----	----	----	----	3	----	2	----	----	----	----	----	----	----
6 to 9 or over:															
Total examined	12	----	----	2	1	2	6	----	----	1	----	----	----	----	----
Number mottled	7	----	----	2	1	2	2	----	----	----	----	----	----	----	----
7 to 9 or over:															
Total examined	14	----	1	2	2	2	2	2	----	1	----	1	----	----	1
Number mottled	3	----	1	1	----	1	----	----	----	----	----	----	----	----	----
8 to 9 or over:															
Total examined	8	----	3	----	1	----	1	1	----	1	1	----	----	----	----
Number mottled	----	----	1	----	----	----	----	----	----	----	----	----	----	----	----

[1] Second group consists of second bicuspids, cuspids, and second molars.

TABLE 4.—*Condition of the enamel of the teeth of 14 persons with third molars erupted, Bauxite, Ark.*

Number of years lived in Bauxite—used municipal deep-well water supply	Age arrived in Bauxite	Present age	Condition of the teeth			
			First permanent group		Second permanent group	Third molars
			Mottled	Stained	Mottled	Mottled
18	4	22	Normal	Normal	Mottled	Mottled.
18	(1)	18	Slightly mottled	do	Slightly mottled	Slightly mottled.
12	7	19	Normal	do	Normal	Mottled.
11	6	17	do	do	do	Slightly mottled.
10	8	18	do	do	do	Mottled.
8	12	20	do	do	do	Do.
8	10	18	do	do	do	Do.
8	7	15	do	do	do	Do.
7	12	19	do	do	do	Do.
7	8	15	do	do	do	Do.
6	12	18	do	do	do	Normal.
2	11	[2] 13	do	do	do	Mottled.
(3)	(3)	17	do	do	do	Normal.
2	13	[4] 15	do	do	do	Do.

[1] Birth.
[2] Now 17 years of age; attended Bauxite school from 13 to 17 years of age; living near Bauxite.
[3] Never lived in Bauxite but attended Bauxite school from 6 to 17 years of age; living near Bauxite.
[4] Now 19 years of age and living in Benton.

was mottled. The third lived in Bauxite from his seventh to nineteenth year. His first and second groups of teeth are normal, but the third molars are definitely mottled. The fourth arrived in Bauxite in his sixth year and has defective enamel of only the third molars. Why these first groups escaped in these two children is not clear. The next eight of these persons came to Bauxite when they were from 8 to 12 years of age. These children show normal enamel of the first and second groups, but defective enamel of the third molars, except in the case of one who arrived there in his twelfth year. One child who attended school in the village from his sixth to seventeenth year, but did not live in the village, had normal enamel. The other one arrived in Bauxite at 13 years of age, and the enamel was normal.

The data seem to indicate that the enamel of the third molars is usually defective if the individual had entered the endemic center any time up to 11 years of age. This uniform peculiarity in the behavior of the development of the enamel of the third molars in individuals who come into these endemic districts after the age of 10 or 11 is considered by us as evidence pointing to the etiology of this condition. As has been shown in previous examinations it can be stated that apparently any enamel in the process of formation during residence in an endemic district will be found to be definitely mottled, due to some influence which operates exclusively in such a district. It seems highly probable that the water and not the food supply of these districts contains the influence responsible for mottled enamel, since we can scarcely assume that an individual coming into such a district suddenly acquires a new (and defective) food habit that deprives the growing enamel of precisely the elements, either in quantity or quality, necessary to its normal development. Such an assumption would imply that each individual had acquired identically the same defective food habit.

TABLE 5.—*Proportion of children with mottled enamed on the first group of teeth hose first group is also stained*

Present age of child	Total number with first permanent group mottled	Number mottled but not stained	Number mottled and stained	Percentage of all mottled cases that are stained also	Percentage by groups
All ages	167	94	73	43.7	
5	1	1	----	----	10.0
6	15	14	1	6.7	
7	24	21	3	12.5	
8	22	17	5	22.7	38.9
9	19	8	11	57.9	
10	14	6	8	57.1	56.1
11	13	4	9	69.2	
12	14	8	6	42.9	
13	14	6	8	57.1	66.7
14	10	4	6	60.0	
15 or over	21	5	16	76.2	

It should also be stated that enamel grown elsewhere (in nonendemic districts) and, hence, normal, undergoes no change whatever so far as observation shows, and certainly it never becomes in the slightest degree mottled, upon exposure in an endemic region.

The question of staining of the defective enamel has not been settled satisfactorily. This should not be confused with the mottling of the enamel, which is definitely known to occur during the developmental period. The percentage of mottled enamel that is stained increases from 10 per cent among 6 and 7 year old children to 67 per cent among children 13 years of age or older. Staining apparently develops and increases with age. No one has ever removed the upper central permanent teeth before eruption, and the question of time of staining will never be definitely settled until this is done. A post mortem would provide the opportunity, but it is a difficult problem to obtain consent for necropsy.

There is no evidence to indicate that in any community has there ever been observed any waning of this deleterious influence through any natural chemical or other alterations of its water supply.

There is the possibility of artificial chemical alteration of the water through certain "treatments" employed commercially, but naturally such a procedure would assume knowledge of the exact chemical nature of the damaging ingredient, and this knowledge is as yet undetermined.

To serve as a check on the children of Bauxite, 124 children were examined in the school at Benton, a town about 5 miles from Bauxite.

Of these 124 children, 103 were native to Benton (or other communities exclusive to Bauxite) and none presented anything but normal enamel. The remaining 21, ranging from 11 to 19 years of age, had formerly lived at Bauxite for longer or shorter periods. Sixteen of this group had lived in Bauxite for 1 year or more, and 11 of these had mottled enamel.

The first, second, and fifth children included in table 6 were born in Bauxite, and left there at 12, 12, and 8 years of age, respectively. The enamel of the teeth of both groups was mottled.

The third and ninth children arrived in Bauxite within the first 2 years of life, and the enamel of the teeth of both groups was mottled.

TABLE 6.—*Mottled enamel of teeth of children living in Benton, Ark., who formerly lived in Bauxite*

Case No.	Number of years lived in Bauxite and used municipal deep well water supply	Age at time of examination	Age on arrival in Bauxite	Age on leaving Bauxite (years)	Condition of the teeth			
					First permanent group		Second permanent group	Third molars
					Mottled	Stained		
1	12	16	Birth	12	Sl. mottled	Normal	Mottled	Not erupted.
2	12	14	do	12	Mottled	do	do	Do.
3	9	11	Under 6 months	9	do	do	do	Do.
4	8	18	7 years	15	Normal	do	Normal	Do.
5	8	13	Birth	8	Mottled	do	Mottled	Do.
6	7	14	4 years	11	Normal	do	do	Do.
7	5	18	do	9	do	do	Sl. mottled	Do.
8	5	17	7 years	12	do	do	Normal	Do.
9	4	19	2 years	6	Mottled	Sl. stained	Mottled	Do.
10	3	15	4 years	7	Normal	Normal	do	Do.
11	2	19	13 years	15	do	do	Normal	Normal.
12	2	16	5 years	7	Sl. mottled	Stained	Mottled	Not erupted.
13	2	16	9 years	11	Normal	Normal	Normal	Do.
14	2	15	7 years	9	Doubtful	do	Mottled	Do.
15	2	13	do	9	Normal	do	Normal	Do.
16	1	15	6 years	7	do	do	do	Do.
17	(1)	12	do	6	do	do	do	Do.
18	(1)	14	5 years	5	do	do	do	Do.
19	(1)	15	2 years	2	do	do	do	Do.
20		13	Unknown	(2)	Mottled	do	Mottled	Do.
21		13	do	(2)	Normal	do	Normal	Do.

[1] Less than 1 year.

[2] Unknown.

Three children (Nos. 6, 7, 10, in table 6) arived at Bauxite at 4 years of age and remained there until 11, 9, and 7 years of age, respectively. These children had normal enamel on the first group of teeth, but the second group was mottled.

The 12th child arrived at 5 years of age and left at 7. The enamel of the first group of teeth was slightly mottled, but that of the second group was definitely so.

Four children (Nos. 4, 8, 14, and 15) arrived at 7 years of age and left at 15, 12, 9, and 9 years of age, respectively. Only one of these (No. 14) had mottled enamel. This was a typical case, the enamel of the first group of teeth being doubtful, but that of the second group was definitely mottled.

The other children had been in Bauxite only 1 year or less, or the time was unknown, and had normal enamel.

McKay, without previous information, was able during the examination to pick every Bauxite child, by reason of the defect, from among the Benton children.

The effect of Bauxite environment on the enamel development in families, shown in table 7, is of interest.

One family with four children, aged 17, 15, 13, and 8, shows the following history:

The 8-year old was born in Bauxite, but had been away 19 months after he was 1 year old. The incisors showed an interesting distribution of the mottling in that in the central portion of the teeth, from mesial to distal, was a band of normal enamel bounded above and below by a mottled area reflective of the periods spent in and away from Bauxite.

The 13-year old child was born in Bauxite and showed mottled enamel of all her permanent teeth, with slight staining of the upper centrals.

The 15-year old came to Bauxite at 2 years of age, left when he was 8 years old, and returned when he was 10. All his permanent teeth were mottled and the upper centrals were stained.

The oldest child is now 17. He entered Bauxite when 4 years old, left at 10, and returned at 12 years of age. His first group of permanent teeth were normal, having grown the enamel before coming to Bauxite. The second group, the enamel of which was grown in Bauxite, was definitely mottled.

Another interesting case is that of a girl of 12 years of age who came to Bauxite between 3 and 4 years of age. The enamel of the first group showed a slight band of mottling near the gums, the remainder of the tooth being normal, and the enamel of the second group was typically mottled. The band of mottled enamel on the first group was grown in Bauxite.

The extraction of a loose temporary molar from the mouth of a native Bauxite child afforded an opportunity of observing that the underlying bicuspid was typically mottled prior to any exposure to mouth conditions.

The data gathered in this survey indicate that there is a specific agent or condition in the environment of the village of Bauxite which interferes with the development of the enamel of the permanent teeth and is but a repetition of the information collected by McKay in his previous studies. This agent or condition is strictly limited to the village, because children in the immediate vicinity of the village are not affected. It does not seem probable that this agent can be in the food, because the mode of life and the diet of these people are the same in the village as outside the village. It attacks the children of well-to-do families as well as those of lower economic status. Physical condition seems to be of no importance; as far as can be seen the only requirement is the use of water from the central deep well supply.

The evidence brought out by this examination at Bauxite, together with similar evidence as to other districts, was responsible for the taking of immediate steps for the abandonment of the then existing deep well supply and the substitution of another source. The absence of this enamel defect in the children native to the neighboring town of Benton led to the drawing upon its source of water supply, which is the Saline River, and adapting it to the domestic needs of Bauxite. This involved piping the river water about 4 miles and passing it through a filtration plant before turning it into the distributing pipes. The change was effected shortly after the time of this study; and it is pointed out, as a matter of interest, that Bauxite is apparently the second community in the world to abandon an otherwise satisfac-

TABLE 7.—*Mottled enamel of teeth of children of the same family, Bauxite, Ark.*

	Number of families having the specified number of children who were examined					
	One child examined	Two children examined	Three children examined	Four children examined	Five children examined	Six children examined
No children with mottled enamel	82	32	13	5	1	1
One child with mottled enamel	69	4	4	2		
Two children with mottled enamel		30	3	5	1	
Three children with mottled enamel			7	3		
Four children with mottled enamel				3		
Five children with mottled enamel						1

tory water supply and substitute another solely because it apparently caused this dental defect. The first community to make such a change was Oakley, Idaho, an acount of which was given in *Dental Cosmos*, September, 1925.

The fairly stable populations of these localities will, within 6 or 7 years, afford an opportunity for observing the influence of the new water supply upon enamel grown subsequent to the change and determine the validity of the indictment of the former supply. No other material changes in the food habit or mode of living are likely to occur. A second survey will be undertaken at an appropriate time in each of these two communities.

Following is a report of analyses of samples of both the old "deep well" water and the new "filtered" water of Bauxite made by the Division of Chemistry of the National Institute of Health.

Surgeon Kempf requested that these waters be examined particularly for manganese. Traces of manganese were found in both of these samples of the order of magnitude of approximately 0.04 parts per million.

The analyses for the more common constituents resulted as follows:

	Deep well water	Filtered water		Deep well water	Filtered water
	Parts per million	*Parts per million*		*Parts per million*	*Parts per million*
Total residue on evaporation	1,003.0	86.00	Iron and aluminium oxides	1.0	0.3
Loss on ignition	43.0	14.00	Calcium (Ca)	25.3	17.6
Fixed residue	960.0	72.00	Magnesium (Mg)	7.0	2.1
Chloride (Cl)	415.9	3.75	Sodium (Na)	344.6	9.6
Sulfate (SO_4)	39.6	15.70	Potassium (K)	9.2	3.4
Nitrogen as nitrate (NO_3)	.3	.03	Alkalinity (phenolphthalein)	1.0	.0
Silica (SiO_2)	18.6	6.00	Alkalinity (methyl orange)	213.7	52.0

The determination of the manganese content of these waters is held to be of value with regard to the possibility of its being the factor accounting for the "brown stain" as noted previously in this report. It is not thought to be associated with the general disruption of the orderly or normal arrangement of the integral enamel structure as indicated by the term "mottled."

It will be noted that there is a decided difference in the chemical structure of these two waters, principally in that the "deep well water" is a sodium bicarbonate water while the "filtered water" is a calcium bicarbonate water, as computed by Mr. W. D. Collins, chemist in charge, quality of water division, U.S. Geological Survey.

The table given below is taken from an article published in *Dental Cosmos*, in 1916, by Black and McKay, showing analyses of waters from various endemic districts studied at that time and is inserted here for purpose of comparison.

Table of analyses of water from various endemic districts

[Given in parts per million]

Constituents	Mine water at V	Lawson Ranch well water	Schole's well	Mine water at E	Single Ranch spring	City water at L	City water at B
Sodium	276.77	207.80	242.70	37.12		3.30	6.21
Potassium		3.35	6.44		11.05		
Calcium	89.75	31.80	33.31	74.50	21.32	5.70	23.44
Magnesium	27.36	37.75	33.60	16.71	5.41	1.70	Tr.
Iron	Nil		3.35	Tr.		.49	1.95
Aluminum	2.74						
Chlorin	47.37	31.00	51.00	14.17	8.31	7.29	4.82
Sulphuric acid	651.49	142.40	250.00	104.66	46.80	7.10	
Carbonic acid	216.78	615.00	530.00	239.60	22.80	15.30	
Silicic acid	16.68	21.65	18.12			7.50	6.45

These analyses throw no light whatever on the probable causal agent. The enamel defect occurs where waters of extremely low as well as of extremely high mineral content are used.

It would seem logical and would be highly desirable that animal experimentation be undertaken in some pronounced endemic district under strict water and dietary control, which would be expected to indicate definitely whether or not the water carries some deleterious agent, or lacks something necessary for the normal growth of the enamel. In this respect it is again brought out as in previous publications by one of us (McKay), that thus far in this investigation this lesion of the enamel had never been reliably reported as occurring in animals.

Various animal feeding experiments have been conducted by Howe and Pierle and others, in which structural damages have been brought about in the bony and certain dental tissues by severe dietary restrictions in the experimental animals, but the reproduction of this specific lesion of the enamel in any of these experiments has, to our knowledge, never been accomplished.

There is some question, then, as to what animal could be most successfully used for this purpose. Kempf suggests the pig; possibly the dog. Without any definite indication it would seem that the experimental animal would best be one in which the period of enamel growth was projected over a period of time corresponding as nearly as possible to that of the human.

No definite conclusions can be deduced at the present time other than that this enamel dystrophy ocurs in certain areas in the United States, and the etiological factors seem to be definitely associated with the water supply of these areas. Exposure of a child, during permanent enamel growth, to the environmental factors in an endemic area appears almost certain to result in the development of mottled enamel.

The findings in this report have been compiled largely by one (Kempf) who has approached this survey from the standpoint of the trained investigator into conditions responsible for general human pathology, with no prejudice or influence from the dental standpoint.

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DISTRIBUTION OF MOTTLED ENAMEL IN THE UNITED STATES

By H. TRENDLEY DEAN

INTRODUCTION

This paper reports the results of a survey to determine the distribution of mottled enamel in the United States, a preliminary report (*1*) of this study having previously been made. No reference will be made to a description of the lesion, its histology, or work of recent investigators respecting its etiology. For these phases of the problem the reader is referred to the work of Black (*2*) and McKay (*3*), Williams (*4*), Churchill (*5*), Smith, Lantz, and Smith (*6*), Smith and Lantz (*7*), and Sebrell, Dean, Elvove, and Breaux (*8*). In 1930 McKay (*9*), in an article on geographical distribution, listed a number of areas where mottled enamel had been definitely demonstrated. In addition to those listed by McKay, Ackeroyd (*10*) and Kehr (*11*) report certain endemic areas in New Mexico and Kansas, respectively. A recent publication by Smith and Smith (*12*) reports about 45 towns or rural districts where mottled enamel is endemic in Arizona. For the furtherance of future study on this problem, McKay (*9*) also compiled a complete bibliography on the literature of mottled enamel.

METHOD OF SURVEY

This survey was made in the following manner: Letters were addressed to the secretaries of each of the State dental societies, stating the purpose of the investigation and requesting the names and addresses of the secretaries of the various component, district, or local societies within the State. A questionnaire was then sent to the secretary of each of the component or district societies. The only exception was that no questionnaire was sent to those societies with almost exclusive urban membership, such as New York City, Chicago, or Philadelphia. Of the 415 questionnaires sent to the various component or local societies asking for a report on mottled enamel within the geographical boundaries of their society, 207 replied.

In certain States where organization of district societies does not completely cover the State, or in States where for geological reasons we desired a more complete report than is ordinarily included in a society report, a questionnaire was sent to one or more individual dentists in each county. The apparent discrepancy between the number of areas

QUESTIONNAIRE SENT TO SECRETARIES OF DENTAL SOCIETIES

Dear Doctor:

There exists in about 55 areas in the United States the enamel dystrophy known as "mottled enamel." The permanent teeth erupt presenting a dead white or opaque appearance, sometimes being pitted. In a high percentage of cases these teeth later take on the characteristic "brown stain." This developmental dystrophy is peculiar to the native-born children of an endemic area, or those taken in the area very early in childhood.

We are desirous of knowing whether this condition exists within the geographical boundaries of your dental society, and would appreciate any data which you might furnish us. If your society is meeting in the near future, would you discuss this matter with the members? If NO cases exist, will you return this questionnaire so that we may likewise have that information?

1. Does mottled enamel occur locally?
2. If present, approximate number of cases?
3. If present, what percentage of native-born children and other susceptibles would you roughly estimate, have it?
4. Are cases from any particular area?
   If so, what city, town, or county?
5. If any particular area is affected, what type of drinking water is used? (River, artesian, shallow wells, spring, etc.)

A self-addressed envelope which does not require postage is enclosed for your answer. Any other information which you might be in position to furnish us concerning this problem would be gratefully appreciated.

Respectfully,

H. T. DEAN,
Dental Surgeon, U.S. Public Health Service.

INDIVIDUAL QUESTIONNAIRE SENT TO DENTISTS, HEALTH OFFICERS, AND PHYSICIANS

Dear Doctor:

There exists in about 75 areas in the United States the enamel dystrophy known as "mottled enamel." The permanent teeth erupt, presenting a dead white or opaque appearance, sometimes being pitted. In a high percentage of cases, and in certain areas, these teeth later take on the characteristic "brown stain." This developmental dystrophy is peculiar to the native-born children of an endemic area, or those taken in the area very early in childhood.

We desire to know whether this condition exists within the geographical boundaries of your county and would appreciate any data which you might furnish. If no cases exist, will you kindly return this questionnaire stating that the county is negative for mottled enamel?

1. Does mottled enamel occur in your county?
2. If present, approximate number of cases?
3. If present, what percentage of native-born children and other susceptibles, would you roughly estimate, have it?
4. Are cases from any particular area?
   If so, what city, town, or part of the county?
5. If any particular area is affected, what type of drinking water is used? (River, artesian, shallow wells, spring, etc.)
   (In the light of recent research on this problem, the drinking water used from birth to about 12 years of age, or during the period of calcification of the permanent teeth, is apparently an important etiological factor.)

Any other information which you might be able to furnish us, either with respect to your own county or some endemic area that you know of, would be gratefully appreciated. A self-addressed envelope which does not require postage is enclosed for your answer.

Respectfully,

H. T. DEAN,
Dental Surgeon, U.S. Public Health Service.

stated in the two questionnaires is due to the fact that the later questionnaire included areas made known subsequent to the date of the first questionnaire. These States included Mississippi, New Mexico, Nevada, Utah, and Wyoming. Areas partially covered by these county questionnaires were certain parts of north central Illinois, where the water obtained from deep wells and used for domestic purposes showed a high sodium bicarbonate content, the Panhandle and west Texas, and rock phosphate areas in Kentucky, Idaho, South Carolina, and Tennessee. This same type of county questionnaire was also sent into certain limited areas in Alabama, Florida, Indiana, North Carolina, North Dakota, Oklahoma, Oregon, South Carolina, South Dakota, and Virginia. In cases where Polk's Dental Register (1928) failed to show a dentist practicing in a county, the questionnaire was sent to the county health officer, and in a few cases to local physicians. In all, 1,197 of these individual questionnaires were sent, and from these, 632 replies were received, while 47 more were returned marked "unclaimed", "deceased," etc. (table 1). The distribution of individual questionnaires is shown in figure 1.

Opportunity is taken here to thank the many general practitioners who cooperated so freely in this survey. Many, in addition to answering the questionnaire as completely as possible, included long reports of local conditions, analyses of water supplies, and summaries of school surveys, revealing the presence or absence of mottled enamel. But for the whole-hearted interest of these individual dentists, physicians, county health officers, and dental society officers who gave so freely of their time and effort, much of this report could not have been written.

Evaluating a survey of this type is fraught with difficulties. First, all reports which were in the least doubtful were eliminated. Often the answer indicated that the individual was unfamiliar with mottled enamel and was confusing it with the hypoplasias concomitant with the erythematous or febrific diseases of childhood. These were of course eliminated from further consideration. Second, those reporting a very low incidence were likewise set aside as unimportant. On the other hand, when several men in different parts of a county call attention to a particular area where mottled enamel is probably endemic, or when local dentists or societies submit extensive reports of local conditions, the evidence becomes of value pending confirmation. It has been impossible to confirm all of these reported areas by personal surveys, but they are included in this paper as "reported areas" so that others interested in mottled enamel may confirm or disprove them by surveys (fig. 2).

Detailed surveys made by the writer included Courtland, Va.; Minonk and Fairbury, Ill.; Windsor, N.C.; Conway, S.C.; rural districts in Berkeley and Charleston Counties, S.C.; and two rural areas in Hickman and Maury Counties, Tenn. In detailed surveys the clinical findings are first recorded, and then the individual water history is noted on a card (fig. 3) provided for that purpose. Of the areas just referred to, Courtland (*3*) and (*13*) and Minonk (*14*) have been previously reported in the literature. In addition to the foregoing, endemic mottled enamel has been observed by the author at Harrold, Miller, St. Lawrence, and Vayland, S. Dak. Time did not permit, however, the taking of a detailed history of each affected individual in order to determine the local incidence. A moderately severe type of mottled enamel with brown stain quite common was observed at Harrold and St. Lawrence.

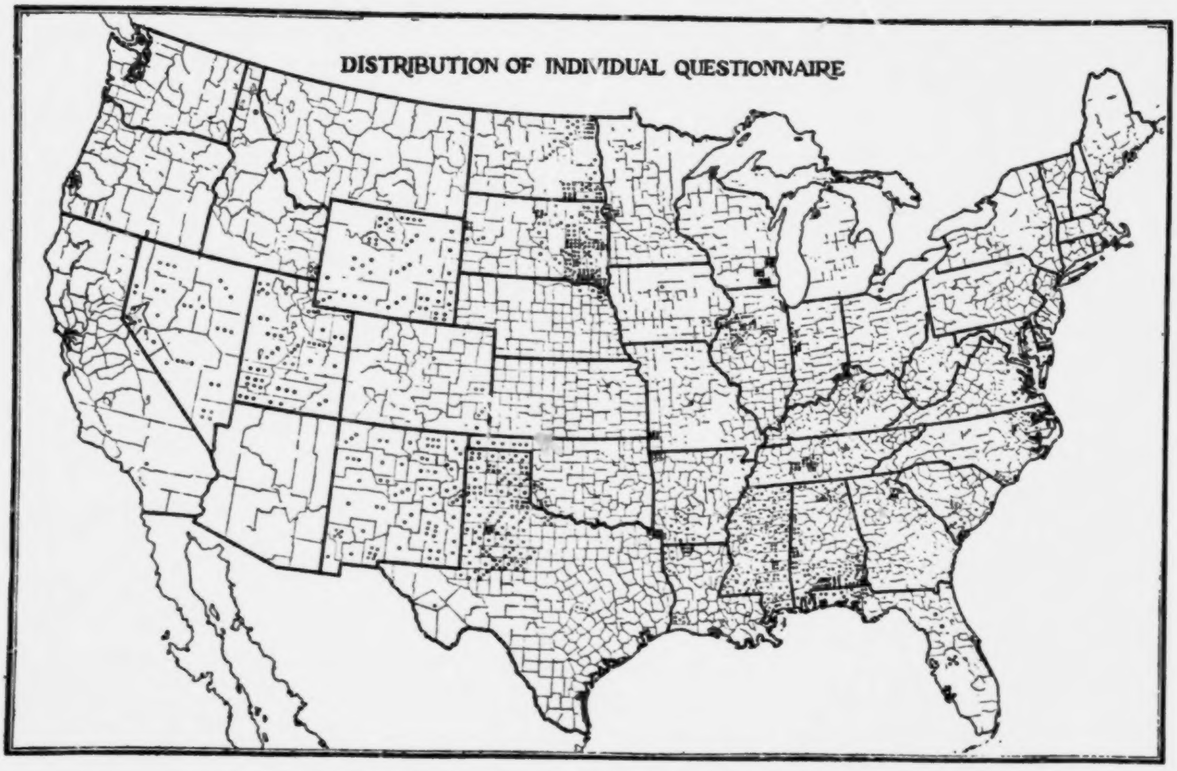


FIGURE 1.—*Map showing distribution of individual questionnaire.*

Table 1.—*Distribution of questionnaires*

State	To component dental societies			To individuals		
	Number sent	Number of replies	Number returned "unclaimed," "wrong address," etc.	Number sent	Number of replies	Number returned "unclaimed," "deceased," etc.
Alabama	3	1	0	109	45	2
Arizona	2	1	0	0	....	....
Arkansas	5	4	0	18	11	0
California (northern)	15	8	1	0	....	....
California (southern)	11	8	0	2	2	....
Colorado	10	5	0	6	2	2
Connecticut	6	5	0	0	....	....
Delaware	1	1	....	14	8	0
Florida	5	4	0	26	12	1
Georgia	12	8	0	11	7	2
Idaho	3	2	1	6	2	0
Illinois	24	12	0	68	38	4
Indiana	14	9	0	31	16	0
Iowa	10	4	0	2	1	0
Kansas	9	3	0	0	....	....
Kentucky	9	4	0	9	7	0
Louisiana	7	3	0	18	6	0
Maine	6	2	0	0	....	....
Maryland	5	2	0	0	....	....
Massachusetts	4	0	0	0	....	....
Michigan	14	2	0	0	....	....
Minnesota	7	3	0	16	11	0
Mississippi	4	2	0	109	54	
Missouri	11	5	0	10	5	6
Montana	10	6	0	0	....	....
Nebraska	8	5	0	4	0	0
Nevada	0	....	....	35	15	2
New Hampshire	3	1	0	0	....	....
New Jersey	12	4	1	0	....	....
New Mexico	0	....	....	74	49	3
New York	7	3	0	0	....	....
North Carolina	5	2	0	38	16	1
North Dakota	6	4	0	56	27	3
Ohio	24	13	0	0	....	....
Oklahoma	6	4	0	15	12	0
Oregon	9	5	0	23	10	3
Pennsylvania	19	12	1	0	....	....
Rhode Island	0	....	....	0	....	....
South Carolina	4	2	0	20	8	1
South Dakota	5	1	0	142	78	4
Tennessee	10	1	0	32	15	1
Texas	19	11	0	146	93	8
Utah	4	3	0	52	26	3
Vermont	3	2	0	0	....	....
Virginia	10	6	0	17	10	0
Washington	13	6	0	0	....	....
West Virginia	12	6	0	0	....	....
Wisconsin	27	11	0	36	21	....
Wyoming	2	1	0	52	25	6
Total	415	207	4	1,197	632	47

SPECIAL POINTS OF INTEREST BY STATES

Arizona

Some of the earliest reports in the literature, Rodriguez (*15*) and McKay (*3*), refer to mottled enamel areas in this State. A recent publication by Smith and Smith (*12*) covers in detail the distribution of mottled enamel in Arizona and lists the affected communities. In this report they state: "The survey disclosed about 45 towns or rural districts in Arizona in which mottled enamel is endemic," in degrees varying from "very mild" to "severe." Most of these communities lie along two rivers, namely, the Gila and the San Pedro, the latter flowing into the Gila.

In addition to these areas, Dr. Leslie W. Foster, field dentist of the Indian Service, writing from Keams Canyon, calls attention to the presence of mottled enamel among the Indian children at the Polacca Day School located at Polacca, in Navajo County. Dr. Fred B. Bass, also a field dentist of the Indian Service, writes from San Carlos that the "condition is very prevalent among all the Indians of southern Arizona, San Carlos Apaches, Pimas, Papagos, and Mohaves. The Apaches live in Gila and Graham Counties, the Pimas in Pinal and Maricopa Counties, the Papagos in Pima County, and the Mohaves in Yuma County." Dr. Bass states that, with the exception of the Papagos, the drinking water is obtained, in the main, from shallow wells, 20 to 40 feet deep. He also adds that mottled enamel is prevalent among white people living on the Gila and San Pedro watersheds.

Arkansas

In 1930 Kempf and McKay (*16*) reported the results of a study made at Bauxite, where mottled enamel was endemic. In addition to this well-known area at Bauxite, Saline County, Gurdon and Okolona in Clark County have been reported to us in questionnaires as endemic areas. Three different dentists call attention to the Gurdon area, two of the reports containing much supplemental data.

California

McKay (*9*) reports mottled enamel at Benton Hot Springs, in the upper Owens River Valley, Montecito, and Las Vegas, suburbs of Santa Barbara; Elsinor Hot Springs, Warner's Ranch, north of San Diego; and Maricopa. With the exception of Maricopa, the use of water from a hot spring is reported as the associated factor. The number of people affected is apparently negligible. With respect to Maricopa, McKay states: "No definite information available either as to extent of the affliction or water conditions." Dr. Clayton W. Akers, formerly of Maricopa, but now practicing at Taft, states that the majority of the children who were born in Maricopa or who moved there at an early age have mottled enamel, some cases being very severe. He adds that the population in the affected area uses a spring for its drinking water.

Colorado

Colorado Springs is the first area in the United States where mottled enamel was reported as endemic. In 1910 Fynn (*17*), referring to the enamel defects of the children of Colorado Springs, stated that "87½ percent of the children born and raised in this city have defects of the enamel." He referred to the condition which we now term mottled enamel as "enamel defects of local origin." This mottled enamel area at Colorado Springs had been the subject of study even before this. There is a reference in the literature by McKay (*3*) that a "systematic endeavor to investigate this lesion was undertaken by the Colorado Springs Dental Society soon after its organization in 1902." McKay adds that the work of investigation began in an organized way in 1908. Black became interested in the problem about this time and visited the area for field study in 1909. This investigation of Colorado Springs and the Pikes Peak watershed by Black (*2*) and McKay (*3*) culminated in a thorough and extensive report on the histopathology and epidemiology of mottled enamel. Williams (*4*) has somewhat modified Black's original concep-

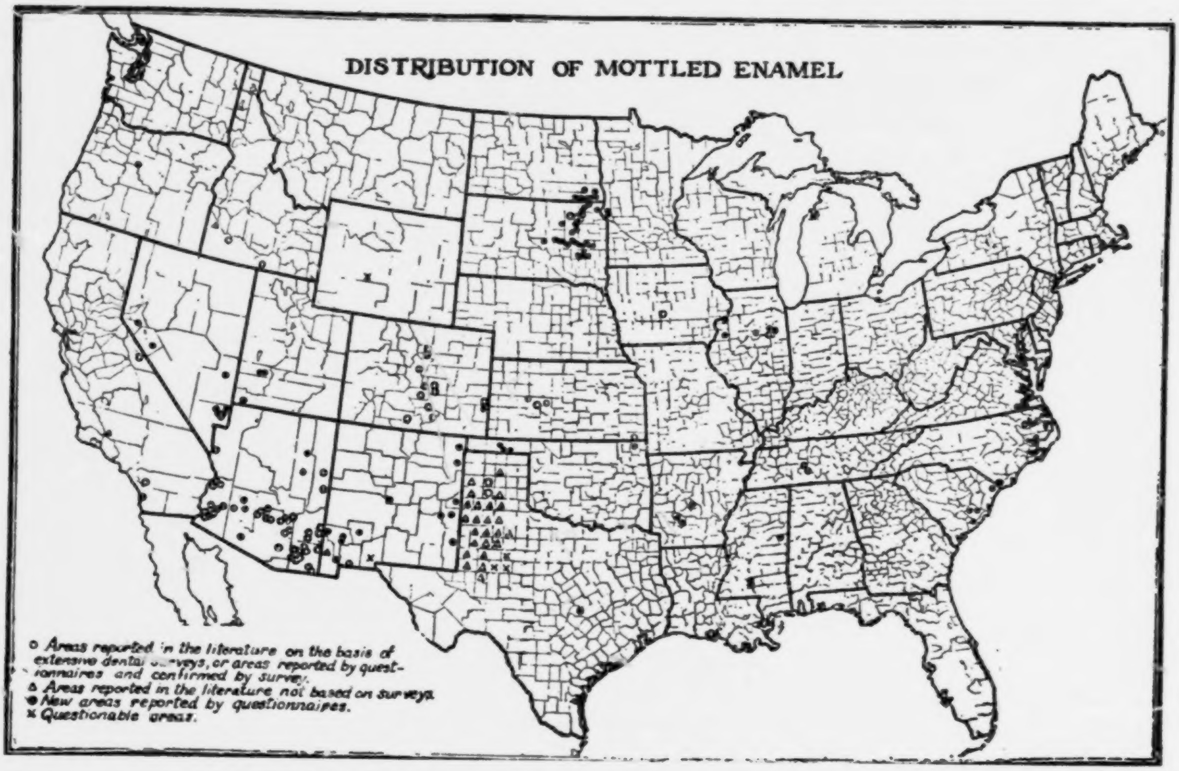

DISTRIBUTION OF MOTTLED ENAMEL
○ Areas reported in the literature on the basis of extensive dental surveys, or areas reported by questionnaires and confirmed by survey.
△ Areas reported in the literature not based on surveys.
● New areas reported by questionnaires.
× Questionable areas.

FIGURE 2.—*Map showing distribution of mottled enamel in the United States.*

tion of the histology of mottled enamel, but subsequent surveys have tended to add further evidence to McKay's original hypothesis that some rare element in the drinking water was the etiological factor. Later McKay (*18*) reported mottled enamel endemic at Towner, a small community in Kiowa County near the Kansas State line.

No new areas were reported in the questionnaires sent the several component societies in the State. Dr. Oren V. Shaw writes that in the examinations made at the Colorado Springs Public School Dental Clinic during the years 1930-31, there were 160 children examined who were born in Colorado Springs. Of this group, 97, or slightly over 60 percent, showed mottled enamel.

Six questionnaires were sent to dentists in Cheyenne, Prowers, and Baca Counties, in the southeastern portion of the State. Dr. Alfred W. Leonard, of Holly, calls attention to a mottled enamel area at Hartman, in Prowers County, where he states that approximately 75 percent of the children are affected. He further adds that the drinking water is obtained from wells, 100 to 300 feet in depth.

IDAHO

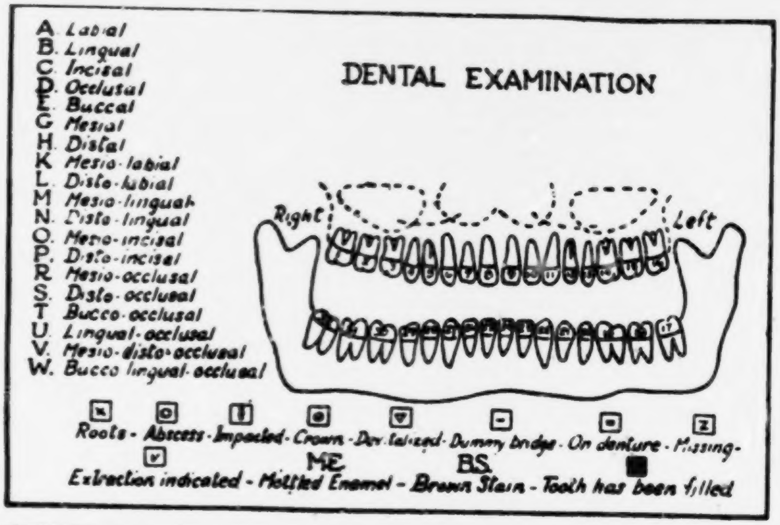
McKay has reported two endemic areas in Idaho, one (*19*) at Oakley in Cassia County, and one (*18*) at Bruneau in Owyhee County. A questionnaire from Dr. Vaughan D. Lyons, secretary of the Upper Snake River Dental Society, indicates that mottled enamel is probably endemic at Wilson, in the northwest section of Owyhee County, and apparently associated with the use of artesian water. Previous reference has been made to this area at Wilson by Smith and Smith (*12*).

ILLINOIS

Minonk, located in the northeastern portion of Woodford County, was reported as an endemic area in 1928 by Bunting (*14*). McKay (*18*) also refers to this area and notes a deep-well association. During February 1932 the writer examined 39 children at the Minonk school, of which number 21, or 53 percent, showed mottled enamel. There were nine children who had used the Minonk municipal water exclusively from birth, five of whom showed mottled enamel. The city water at Minonk is obtained from two deep wells. The "old" well is 1,850 feet deep while the "new" well is reported to reach a depth of 2,005 feet.

Fairbury, a community with a population of 2,310, and located in the southern part of Livingston County, was reported as an endemic area by the McLean County Dental Society. The author visited this area shortly before the examination at Minonk and examined 68 children at the North School. These children were principally from the sixth and seventh grades. Of this group, 21 had used Fairbury city water exclusively from birth; 14 of these, or 66 percent, showed mottled enamel. Fairbury obtains its municipal water supply from three wells, 2,100, 2,000, and 1,600 feet in depth, respectively.

Dr. L. W. Neber, former superintendent of mouth hygiene of the Illinois State Department of Health, who assisted in the examination at Minonk, and the Rock Island Dental Society report mottled enamel at Milan, a small community with a population of 888 located in Rock Island County. Dr. Neber stated that


A. Labial
B. Lingual
C. Incisal
D. Occlusal
E. Buccal
G. Mesial
H. Distal
K. Mesio-labial
L. Disto-labial
M. Mesio-lingual
N. Disto-lingual
O. Mesio-incisal
P. Disto-incisal
R. Mesio-occlusal
S. Disto-occlusal
T. Bucco-occlusal
U. Lingual-occlusal
V. Mesio-disto-occlusal
W. Bucco lingual-occlusal
DENTAL EXAMINATION
Right
Left
Roots - Abscess - Impacted - Crown - Devitalized - Dummy bridge - On denture - Missing -
ME
BS
Extraction indicated - Mottled Enamel - Brown Stain - Tooth has been filled

LOCATION ______________________ DATE ____________

Name: ______________________ Age: ____ Sex: ______ Color: ________

HISTORY

Residence from Birth in Chronological Order	Duration (Years)	Source of Drinking Water				
		Municipal	Artesian	Surface Well	Spring	Cistern
Birth Place						
1. ____________	____	____	____	____	____	____
2. ____________	____	____	____	____	____	____
3. ____________	____	____	____	____	____	____
4. ____________	____	____	____	____	____	____
5. ____________						

Brothers or Sisters in the same school. B: ______________________

S: ______________________

Remarks: ______________________

FIGURE 3.—*Record of dental examination and individual drinking water history.*

he found a number of children showing the white opaque type of mottled enamel with little or no brown stain. The drinking water is obtained from a 1,100-foot drilled well. The Warren Dental Society reports a low incidence of mottled enamel at Monmouth and adds that the municipal water supply is obtained from two wells 2,445 feet deep and cased 1,200 feet. Later communications from Dr. E. B. Knight, the secretary of the society, indicate that mottled enamel is probably more prevalent than when first reported.

Dr. P. A. Pyper, of Pontiac, reports mottled enamel cases in the surrounding rural districts where deep-well water is used. Both he and Dr. L. B. Muzzy, of Odell, call attention to mottled enamel in Odell. Dr. Pyper also directs attention to several farms around Emington, about 25 miles northeast of Pontiac, where the inhabitants use deep wells and the children born and reared there show badly marked teeth. Dr. W. G. Ross, of Kempton, Ford County, writes that there are many cases in Kempton. It is interesting to note that Kempton is not far from the area referred to by Dr. Pyper around Emington, in Livingston County.

IOWA

Mottled enamel is reported as endemic at Ankeny, in Polk County, by Ostrem, Nelson, Greenwood, and Wilhelm (*20*). The water supply, according to these investigators, is obtained from deep wells.

KANSAS

Mottled enamel in Kansas has been extensively studied by Kehr (*11*). This investigator reported areas of mottled enamel at Chetopa, La Crosse, Utica, and Bazine, which were confirmed by survey.

MINNESOTA

McKay (*19*) reports an area at Graceville, Big Stone County, and definitely associates it with the use of deep well water. Dr. W. H. Strong, of Graceville, states that the wells from which the municipal water supply is obtained are from 500 to 600 feet deep. Dr. Strong also estimates that about 90 percent of native-born children are affected.

Sixteen questionnaires were sent to dentists in Traverse, Stevens, and Big Stone Counties, of whom 11 replied. Dr. Alf T. Watzke, of Brown Valley, Traverse County, writes that, formerly, when the people of Brown Valley obtained their drinking water from artesian wells, mottled enamel was endemic among the children. The community installed a city water supply which is obtained from a spring, and children calcifying their permanent teeth under the new water supply are free from the dystrophy. Dr. Harold N. Weickert, of Wheaton, Traverse County, notes in his report that mottled enamel is endemic in a small section of the southern part of this county where the children use a "soft" water obtained from deep wells.

MISSISSIPPI

Seven areas endemic for mottled enamel are located in that part of Virginia, North Carolina, and South Carolina lying in the Atlantic coastal plain. Mississippi is one of the few States lying wholly in the Atlantic coastal plain. There are no reports in the literature of mottled enamel areas located in this State. The component or district dental societies are limited to four, located principally around the larger centers of population.

A survey by counties was decided upon and one or more questionnaires were sent to practicing dentists of each county. There are 84 counties in this State. Polk's Dental Register (1928) listed no practicing dentist in five counties, viz, Benton, Forrest, Issaquene, Sharkey, and Warren. Questionnaires were sent to one or more individuals in each of the remaining 79 counties. These questionnaires totaled 109, and 54 replies from

46 different counties have been received.

Dr. D. N. Garner, President of the Mississippi Dental Association, writing from West Point, Miss., states: "West Point is located about on the dividing line between the sandy, or hilly section, and the black lime, or prairie belt of the State. Going west from this town into the hills, where the people use water from shallow, or pump, wells, we find no mottled enamel of any consequence. Going east and south into the black land, the condition is very prevalent, some cases having but little enamel on their permanent teeth. In this section people use water from artesian wlls. There are two strata of this water. The first seems to be free from minerals, and will overflow only in low lands. The second seems to have quantities of some mineral, largely iron, and overflows on higher land. This last-mentioned section is where we find so much mottled enamel."

Those counties in which the reports indicate mottled enamel are as follows:

Noxubee County: Doctor Garner writes that he examined about 200 school children at Brookville and found about 40 cases of mottled enamel. Dr. D. W. Reilly, of Okolona, also notes in his report that mottled enamel occurs at Brookville and states that the water supply is obtained from a deep well. Dr. M. M. Brown, of Macon, estimates that there are about 200 cases in the county principally from the eastern and southern sections. He also notes an artesian well association.

Lawrence County: Dr. B. A. Batson, of Monticello, states that a large percentage of the population is affected with mottled enamel. It is interesting to note that Dr. Noel R Thompson, of Brookhaven, also calls attention to a mottled enamel area in Lawrence County, located at Nola.

Nevada

This State is very sparsely populated and there are no district dental societies. It became necessary, therefore, to send one or more questionnaires to dentists in each county. In counties where there were no dentists, information was requested from the county health officer or local physician. Points of interest disclosed in the replies were as follows:

Clark County: This is probably the most extensive area in Nevada. Dr. J. Dayton Smith of Las Vegas writes: "In the Moapa and Virgin valleys, about 80 miles from Las Vegas, the native children have mottled enamel almost 100 percent." Dr. Smith states that the communities affected are Moapa, St. Thomas, Bunkerville, and Overton. He adds that the water supply is obtained from river and shallow wells. Dr. Charles H. Masterson, formerly of Tonapah, now of Las Vegas, also calls attention to the high incidence of mottled enamel in this area and states that most of the cases he has seen were associated with the use of water from shallow wells. Dr. E. H. Conger, of St. George, Utah, makes some interesting comments respecting this area. He has an office in Overton, and one at Mesquite, which he visits bimonthly. He writes that the people of Overton get their drinking water from a small stream known as the Muddy Valley River, the source of which is a large nearby spring. In Mesquite, the people obtain water from the Rio Virgin, into which a number of hot sulphur springs empty a few miles from the community. Dr. Conger states that a large percentage of children in both communities are affected. Dr. Wilford C. Cox, also of St. George, Utah, likewise reports a high incidence of mottled enamel in the Moapa Valley of Nevada.

Lincoln County: From Caliente, Dr. Willard W. Stockham, a physician, writes that about 25 percent of the children at Panaca are affected. He states that they obtain their drinking water from a spring, the water of which is carried in open ditches.

Lyon County: Both Dr. E. B. Dawson, of Yerington, and Dr. C. H. Masterson, formerly of Yerington, but now practicing at Las Vegas, report mottled enamel in this county. The latter noted that a large number of cases came from the "Wabuska district" where the drinking water is apparently obtained from shallow wells.

Mineral County: Dr. Wm. J. Lanahan, field dentist of the Indian Service, while making examinations at Schurz, Nev., observed typical cases of mottled enamel among Indians who had resided at Sodaville from birth until adult life.

New Mexico

The only area of mottled enamel endemicity definitely described in the literature is that at Columbus in Luna County, which was reported by Ackeroyd (*10*) in 1923. McKay (*9*), in an article on geographical distribution, states, in referring to New Mexico, that it is found in "various small communities over the State, principally adjacent to the eastern boundary (Texas). No reliable data as to water supply and no definite knowledge of the particular towns afflicted."

There are no component or district dental societies in the State. Seventy-four questionnaires were sent to individual dentists and replies have been received from 49. Of the 31 counties in the State, one or more replies have been received from 29 counties.

Areas reported are as follows:

Chaves County: Dr. J. E. Rose of Roswell, reports a small area in the extreme northeastern section of the county near Kenna.

Dona Ana County: From the reports of Dr. S. T. Barnhill and Dr. O. H. Brown of Las Cruces, it would appear that mottled enamel is being produced in certain sections of the county where the drinking water is being obtained from shallow wells. The data with respect to this county is indefinite; but Las Cruces, the county seat, where the drinking water is obtained from wells approximately 200 feet deep, is apparently nonendemic for mottled enamel. This county is listed as questionable.

Grant County: Mottled enamel is seemingly widespread throughout this county. Dr. L. A. Jessen, of Santa Rita, states that a high percentage of individuals using certain water supplies along the Membres River are affected. He specifically refers to the Sherman School on the Membres River where a very high percentage of children are affected. Dr. Frank E. Morton, of Hurley, writes that approximately 95 percent of the native-born children are affected. From another section of the county, Dr. W. H. Chapin, of Silver City, reports mottled enamel in the Gila River district in the western portion of the county. Again, from another section of this county, the extreme southern tip, comes a report from Acting Assistant Surgeon E. J. Thornberry, of the Public Health Service, that practically all the children of Hachita develop mottled enamel. Several of these observers supplemented their reports with extensive descriptions of local conditions.

Hidalgo County: Dr. R. E. Buvens, of Lordsburg, states that about 50 percent of the children are affected and adds that this is also true of some of the nearby communities. This area has also been referred to by Smith and Smith (*12*).

Lea County: Dr. H. J. Coburn, of Tatum, states that mottled enamel is general throughout the county and estimates that 90 percent are affected. He states that drinking water is obtained from shallow wells.

Luna County: From Deming, Dr. M. J. Moran, writes that mottled enamel occurs in all the district south of the Florida and Treshermanas Mountains but not in the immediate vicinity of Deming. He estimates that at least 90 percent of all children in this endemic district are affected. He also adds that mottled enamel is still endemic at Columbus.

Roosevelt County: Dr. Raymond W. Means, of Portales, states that a severe type of mottled enamel is widely distributed in this county.

Sierra County: Dr. A. C. White, the county health officer, writes that 40 to 50 percent of the children of this county are affected. There is no dentist listed as practicing in the county.

Torrence County: Dr. E. S. Hamel, who formerly practiced at Mountainair, estimated that about 25 percent of the children are affected and noted that water from deep wells was used for drinking purposes.

Union County: From Clayton. Dr. C. E. Keller, writes that about 50 percent of the children are affected in the town of Amistad, where water for domestic purposes is obtained from shallow wells. Dr. C. M. Hurley, also of Clayton, reports that he finds mottled enamel in those individuals coming from the southern part of the county around Bueyeros and in the northern part around Cuates. Capulin in the extreme northwestern part of the county is considered questionable.

No reports were received from De Baca or Mora Counties; all others were reported as negative.

North Carolina

This survey disclosed two areas in North Carolina where mottled enamel is endemic. One reported by Dr. W. R. Davis, of Whiteville, is a narrow strip, 5 to 10 miles wide, a thinly populated area in Columbus and Brunswick Counties, where these two counties border Horry County, S.C. The mottled enamel here, as in Horry County, is apparently associated with the use of flowing artesian well water. The other area is in Bertie County, located in the northeastern section of the State. The county seat of Bertie County is Windsor, a community with a population of 1,425. The writer made a detailed examination of 132 children of the sixth, seventh, and eighth grades in the Windsor school. Twenty-two of these pupils had used the Windsor municipal water exclusively from birth, and 19, or 86 percent, showed mottled enamel. The Windsor municipal water supply is obtained from deep wells.

Many of the pupils at the Windsor school come from distances as great as 10 or 12 miles. Consequently the examination gave us a picture of mottled enamel as it existed not only in Windsor but in parts of Bertie County as well. In the group examined, there were 70 children who stated that at no time did they live in Windsor. Among these 70 from the rural districts, there were 43 who gave a history of having lived in the same place and used the same water since birth. Sixteen of the 43 showed mottled enamel, 4 moderately severe, while the remainder ranged from mild to very mild. All gave a history of using shallow well water at home. The dug or driven wells in this area vary in depth from 12 to 25 feet. The only deep-well water (artesian) used by these children was that consumed at the Windsor school. The children from the rural districts who show mottled enamel are, in the main, from that section of Bertie County east and northeast of Windsor.

North Dakota

In addition to the town of Lidgerwood, referred to by McKay (*9*), reports from various dentists indicate that mottled enamel occurs in the following counties:

Dickey County: Dr. H. E. Thomas, of Ellendale, calls attention to mottled enamel in this county among the users of artesian water. Dr. H. F. O'Connor, of Oakes, states that probably 90 percent of the children in the eastern part of Dickey County and the western part of Sargent County using artesian water are affected.

Ransom County: Dr. George A. Nelson, of Enderlin, and Dr. Arthur B. Ostrander, of Lisbon, state that mottled enamel is quite prevalent among children on farms where artesian water is used.

Richland County: Dr. R. M. Johnson, of Wyndmere, writes that the majority of the inhabitants of Wyndmere use surface wells and are free from mottled enamel, but on the farms near Wyndmere, where artesian water is used, mottled enamel is being produced in the children. Dr. A. R. Thomason, of Hankinson, reports cases in the western part of the county toward Geneseo.

Sargent County: Dr. K. J. Cole, of Milnor, estimates that 75 to 80 percent of the children are affected, and he associates the condition with the use of artesian water. Dr. E. G. Movius, formerly of Lidgerwood but now of Mohall, also calls attention to this district and states that mottled enamel is endemic at Cayuga and Geneseo.

Oklahoma

Six dentists and a physician report mottled enamel in Guymon, Hooker, and certain sections of the southeastern section of Texas County and the southwestern part of Beaver County. The report of Dr. Carl Puckett, managing director of the Oklahoma Tuberculosis and Health Association and submitted by Dr. T. W. Sorrels, chairman of the oral hygiene and educational committee of the Oklahoma State Dental Society, contains much relevant data respecting mottled enamel in this region. Dr. Puckett also notes an observation made by McKay (*18*). His tabulations indicate a slightly lower incidence of dental caries among those with mottled enamel than is present in a similar school group in another part of the State where mottled enamel is not endemic.

Dr. Hugh Jeter, of Oklahoma City, calls attention to an area at Bluejacket in the northeastern section of the State, where mottled enamel is reported as endemic. Mr. H. J. Darcey, State sanitary engineer, writes that the inhabitants of Bluejacket obtain their domestic water supply from individual wells.

Oregon

Dr. E. E. Gray, secretary of the Central Oregon District Society, reports a small area 32 miles north of Bend, Oreg. Dr. Gray states that the affected area is a small community near Opal City in Jefferson County, known as Opal Springs. In 5 white families there were 3, 3, 2, 1, and 2 cases, respectively, while in 1 Japanese family there were 3 cases of mottled enamel, or a total of 14 cases. All of these 14 were in children who were born there, their ages ranging from 8 to 19 years. Dr. Gray states that the water supply was obtained from a 2,000-foot "railroad" artesian well which was sunk in 1910. This well was used until about 3 years ago. In addition, Smith and Smith (*12*) report an endemic area at Talent.

South Carolina

As in North Carolina and Virginia, mottled enamel in South Carolina is apparently limited to communities lying in the Atlantic coastal plain.

Horry County: Dr. Carl Busbee, president of the Pee Dee Dental Society, reports the city of Conway and Horry County as endemic areas. There is likewise a reference to the presence of mottled enamel in Conway in a recent publication by Smith and Smith (*12*). The writer surveyed this area during April 1932. Conway has a population of 3,011, and its municipal water is obtained from three artesian wells, a 3½-inch well put down in 1896 to a depth of 450 feet, a 10-inch well drilled in 1918 and 400 feet deep, and a 3-inch well drilled in 1924 to a depth of 305 feet. All wells are cased down to the first rock. In addition to the municipal water supply, there are about 30 flowing wells within the city limits. There are likewise many flowing wells in the smaller communities and surrounding rural districts of the county.

One hundred and four children were examined at Conway and 15 at Myrtle Beach. Detailed examination of each tooth, with chronological water history of each individual, was made and recorded as follows:

Group A Entire 6th grade, Conway white school	59
Group B Selected cases from the 8th grade of the Conway Junior High School (individuals who had never lived in Conway)	28
Group C Special Cases	17
Group D Myrtle Beach grade school	15
	119

In group A there were 14 children who stated that they had used Conway water exclusively from birth. All showed mottled enamel, the majority in a moderate to severe degree. An analysis of cases listed under groups B and C indicates that the same factor which is apparently causing mottled enamel among the children in the city of Conway is likewise operative among those individuals from the rural districts who use artesian water during the period of calcification of their permanent teeth. Although statements of previous investigators (*16*) (*6*) indicate that lesions corresponding to human mottled enamel have not been observed in domestic animals of endemic areas, the writer in studying the literature on this subject found that a lesion of the permanent teeth of ruminants, horses, and other animals had been observed in North Africa, where it is known as "Le Darmous" (*1*). In this connection it may be interesting to mention that the writer has succeeded in finding instances of "Le Darmous," or animal mottled enamel, in the United States. An examination of a score or more of cows in Horry County, S.C., indicated that these developmental imperfections may show in the permanent teeth of cows which drink continuously of artesian waters showing a high fluoride content. It was possible to find three cows, about 4 years of age, whose consumption of artesian water from birth had been quite constant. Although the centrals and first laterals showed little other than an abnormal dullness, the second laterals and corner teeth showed definite developmental imperfections which we would be justified in considering the analogue of human mottled enamel. The second laterals and corner teeth, according to Chauveau (The Comparative Anatomy of the Domesticated Animals, by A. Chauveau, revised by S. Arloing; Second English Edition translated and edited by George Fleming. D. Appleton & Co., New York, 1905) erupt at about 3 and four years of age, respectively. In 2 of the 3 cases, children of the household using the same water as did the cows showed a moderately severe mottled enamel. In the third case, there were no children in the house, but the well water on analysis showed considerable amounts of fluorides. Cows imported from other counties (nonendemic areas) did not show this developmental dystrophy. This study is being continued and it is hoped other instances of its occurrence in the United States may be found. A shifting population with consequent broken water histories obviated drawing any inferences from the examinations listed in group D.

Berkeley County: Dr. J. K. Fishburne, county health officer at Moncks Corner, reports that an examination of 11 schools in the county disclosed 297 cases of mottled enamel varying in degree from very mild to moderate. In March 1933 the writer visited this county and observed mottled enamel in two widely separated sections. At the Chicora school, near Cross and the Orangeburg County line, a few mild to moderate cases of white opaque type of mottled enamel were observed. All cases were associated with the use of deep-well water. One family on Route No. 31 near the Orangeburg County line showed a typical mottled enamel involvement. There were 8 children in the family and only the oldest, aged 18, was free from the defect. The youngest, aged 6, showed questionable signs on the few permanent teeth present, while the remaining six children showed definite signs of mottled enamel ranging from very mild to a moderate degree of involvement. The water apparently causing the dystrophy was from a well 93 feet deep but cased down only 36 feet.

In the northeastern section of the county there is a logging community known as Witherbee. About a dozen families are supplied with water piped to their individual homes from a storage tank. The water is obtained from a well 289 feet deep which was put down in 1926. This water is apparently affecting all the children using it, some showing a moderate degree of involvement. The older children show a lesion only on the cuspids, bicuspids, and second molars, the teeth calcified earlier being normal. The younger children show the characteristic manifestations of mottled enamel on the first molars and incisors.

Charleston County: The possible relationship between rock phosphate deposits and mottled enamel will be referred to more completely in that portion of this paper relating to Tennessee. The phosphate deposits of Charleston County have been described in detail by Rogers (*21*), and a survey of certain of these areas in the northern part of the county was made with the assistance of Dr. Leon Bonov, the county health officer. Seven cases of very mild to mild, white, opaque mottled enamel were observed among colored children using water from two shallow wells in these phosphate areas. These two wells are located between Johns Island and Lambs. The evidence relative to mottled enamel among users of shallow wells in this particular rock phosphate area is indefinite, and conclusions are apparently not justified by the evidence available at the present time.

Mottled enamel associated with the use of water from deep wells is, however, not uncommon in this county. At the Red Top colored school near the Johns Island railroad station, 13 cases among the 54 children examined were noted. Many of these were moderate to severe in degree of involvement, a few showing brown stain on the incisors. Eight gave a history of drinking water from the artesian well at the Johns Island railroad station. The others were found to be associated with water from two other nearby deep wells. Other examinations in various schools indicated that other deep wells in the rural districts of the northern part of this county were likewise producing mottled enamel, but none as severe as noted around Johns Island.

SOUTH DAKOTA

Areas of mottled enamel in South Dakota were reported as early as 1918 by McKay (*22*), who demonstrated its presence by a survey at Britton, Kidder, Langford, Pierpont, Andover, Groton, and Aberdeen, all apparently associated with the use of artesian water during the period of calcification of the permanent teeth. Five questionnaires were sent to the district dental societies in the State. One reply, from the Second District Dental Society, has been received. In addition, questionnaires were mailed to 142 individual dentists in 29 different counties. Seventy-eight replies have been received from these individual dentists, many of them supplying much supplemental data. Many of these questionnaires were sent to dentists solely on the basis of the type of water used in their communities, which, in turn, were based on the geological reports of Darton (*23*).

A report by counties follows:

Beadle County: Dr. George A. Youel, of Huron, writes that mottled enamel is not endemic among the children of Huron, which uses a filtered river water for its municipal supply. Dr. Youel calls attention, however, to mottled enamel in areas using artesian water and particularly refers to Cavour, Yale, Iroquois, and nearby farms. Several other dentists of Huron call attention to mottled enamel in the northern half of the county bordering on Spink County. Dr. A. A. Wollman particularly calls attention to the neighborhood around Hitchcock.

The writer examined 63 children in the school at Wessington. There was one mild case of mottled enamel and four cases very mild to questionable. The one mild case was in a child from a nearby country district who had used artesian water exclusively from birth. At Wolsey, the school was not in session and the writer examined 14 children on the street. There were four moderate to severe cases of mottled enamel generally associated with the use of the artesian city water.

Bon Homme County: Six replies indicate that this county is apparently negative.

Brookings County: Six replies from dentists indicate that this county is negative.

Charles Mix County: Four replies from this county fail to point to any outstanding area. One dentist reports mottled enamel around Wagner, but the information at present is indefinite. This county is being listed as negative pending further information.

Davison County: Replies from seven dentists in Mitchell indicate that this county is nonendemic for mottled enamel.

Faulk County: Dr. Max H. Moore, of Faulkton, is of the opinion that the mottled enamel cases being produced in this county are, in the main, from around Chelsea, in the northeastern part of the county.

Hand County: The writer examined the pupils of four schools along U.S. Route No. 14 in this county. At Ree Heights 50 children in the fifth, sixth, seventh, and eighth grades were examined. Only one case of mild mottled enamel was observed, and in this case there was a mixed water history. At the Spears school, midway between Ree Heights and Milier, 14 children were examined. Eight of the 14 showed a mild degree of white, opaque mottled enamel associated with the use of artesian water. At Miller, 102 children in the sixth, seventh, and eighth grades were examined. Thirty-eight cases of mottled enamel were observed, practically all of which were of a mild type. At St. Lawrence, 46 children in the fifth and sixth grades were examined, of whom 19 showed mottled enamel. Seven of the 19, principally from the country districts, showed mottled enamel in a moderate to severe form. Two other individuals in the high school who had used water from artesian wells on the outskirts of the community showed a severe form of mottled enamel. The characteristic brown stain was present in many of these moderately severe cases. At Vayland 11 cases of mottled enamel were observed among the 25 persons examined. These were all of a mild type, and no brown stain was observed.

Hughes County: The children in five schools, 76 children, from Pierre to Harrold were examined. In the one mild case observed in this entire group the affected teeth had apparently calcified while the child was living in Lyman County, where mottled enamel is occasionally observed in children living on ranches where deep well water is used. At Harrold, however, an examination of 58 children showed 9 cases of mottled enamel, varying from moderate to severe, several showing marked brown stain. The mottled enamel and brown stain observed at Harrold was similar to that observed at St. Lawrence and more severe than any noted in the various schools along Route No. 14 between Pierre and Wolsey.

Kingsbury County: Dr. P. L. Scofield of De Smet reports a high incidence of mottled enamel in the western third of the county associated with artesian water. Dr. W. C. Gross reports a high percentage of children affected in Iroquois, with many showing the characteristic brown stain. Dr. M. G. Jensen of Lake Preston reports a high incidence of mottled enamel in Lake Preston associated with the use of artesian water. Bancroft, in this county, has also been reported as an endemic area.

Minor County: Dr. Leo V. Schueller of Howard states that mottled enamel is very prevalent in the western half of the county and particularly refers to Fedora, Roswell, and Vilas. Dr. Albert S. Bensend, of Carthage, reports that a high percentage of children in Carthage are affected and adds that the condition is also present in Esmond and the western part of the county.

Roberts County: Dr. A. R. Sorbel and Dr. J. A Robertson, both of Sisseton, write that there are areas where mottled enamel is endemic in this county. Dr. Robertson further calls attention to the fact that Roberts County is composed of hills to the west which rise to a height of 2,000 feet and the Whetstone Valley to the east. Many artesian wells have been sunk in the Whetstone Valley during the past 20 years and water is obtained at depths of from 700 to 900 feet. Doctor Robertson notes that children drinking artesian water in the valley invariably show mottled enamel, while those from the hill country, although separated by only a few miles, are not affected.

Sanborn County: Dr. D. St. I. Davies, of Woonsocket, makes some interesting comments relative to Woonsocket and neighboring communities going back over a period of 50 years. He states that when the county was first settled and the inhabitants depended on creeks or shallow wells, near sloughs, there was no mottled enamel. Then in the early 90's deep artesian wells (around 700 feet deep) were put down. Water from these wells produced mottled enamel. He further adds that within recent years, so-called "soft water" wells (about 200 feet deep) have been used more and more and children calcifying their permanent teeth while using this water are free from the defect. Doctor Davies has offices both in Woonsocket and Artesian and states that mottled enamel is more frequently observed in Artesian than in Woonsocket, where the gradual change in the water supply has almost eliminated it. Dr. Leo V. Schueller, of Howard, also refers to Forestburg, between Woonsocket and Artesian, as an endemic area.

Spink County: Dr. Clyde A. Peterson, of Doland, writes that approximately 50 percent of the children show mottled enamel, associates it with the use of artesian water, and localizes it in the eastern part of the county. From Dr. V. P. Poulson, of Conde, comes another interesting report. He states that a high percentage of individuals are affected wherever artesian water is used and especially reports its presence in the northern section of the county where Conde is located. From Redfield, in the western part of the county, and a little to the south, Dr. Earl F. Harrington writes that he sees only a few cases and these apparently from the northern section of the county. Dr. W. G. Gross, of Iroquois, states that mottled enamel is endemic at Frankfort.

TENNESSEE

No reply was received from the query sent to the secretary of the Tennessee State Dental Association, and questionnaires were sent to the secretaries of the 10 component societies as listed in Polk's Dental Register (1928). In answer to these 10 requests for information, one reply was received. This was negative.

In view of the extensive deposits of natural phosphates in the State, further study was deemed advisable. Certain rock phosphates, according to Marshall, Jacob, and Reynolds (*24*), show a fluorine content as high as 3 or 4 percent. These investigators found that a number of samples from Tennessee contained more than 3 percent. A report by Waggaman (*25*) gives a detailed description of the location of the Tennessee natural phosphates, while other work of interest in connection with this study is that of Carter (*26*) relative to the solubilities of inorganic fluorides in water.

Two counties in Tennessee, Maury and Hickman, were visited. At Mount Pleasant the two dentists stated that they see mottled enamel at times and that the cases are apparently from the rural sections. The municipal water supply of Mount Pleasant is brought a distance of about 10 miles, and its source is in a limestone region. About a quarter of a mile, however, from Mount Pleasant there is a small settlement of a dozen or more colored people who obtain their drinking water from a spring which comes out through a phosphate formation Six children who had used water from the spring during the period of calcification of their permanent teeth were examined. Four showed a "mild" degree of involvement of the white opaque type of mottled enamel. One Negro girl, age 11, who had used this spring for the past 3 years only, showed definite markings of mottled enamed on all of the bicuspid teeth, while the remaining teeth were normal. A mining community about 5 miles from Mount Pleasant was next visited. The water supply of this community is obtained from a well 65 feet deep and reported as cased its entire depth. The natural phosphate deposits in this particular area are about 20 feet deep and the community water supply is apparently obtained from a source considerably below the phosphate deposit. No mottled enamel was observed in about a dozen children examined.

In Hickman County, a small mining settlement at Twomey was visited. The water supply there is obtained from a spring known as "Twomey Springs" which issues from a phosphate deposit. The mining superintendent stated that this particular phosphate was high in fluorine. In one family there were three children who had used this spring exclusively from birth. Their ages were 8, 10, and 13, respectively. All showed a mild type of white, opaque mottled enamel, one in addition showing the characteristic brown stain on the superior incisors. Another family with a child aged 10 had moved into the community 3 years previously. The second bicuspids of this child showed mottled enamel while all other teeth were normal. A fifth child, aged 13, gave a mixed history of drinking water, having used water from both Twomey Springs and a surface well. This child showed questionable markings on the central incisors and one bicuspid while all the remaining teeth were apparently normal.

The finding of mottled enamel in children who had used water passing over or through rock phosphate deposits is of interest. Velu (*27*) reports a similar condition in North Africa known as "le darmous." In the opinion of Velu, "le darmous" is caused by the ingestion of small amounts of fluorine present in the drinking water as a fluoride, due to its passage over or contact with the beds of natural phosphates. "Le darmous" has been studied during the past 10 years at the Institute of Pasteur in Algiers, and their animal experiments and investigations become of value in connection with the study of mottled enamel. From Velu's description, illustration, epidemiological data, and apparent causative factor, we infer that "le darmous" and mottled enamel are identical.

TEXAS

In 1916, McKay (*3*) called attention to certain mottled enamel areas in Texas. Pierle (*28*) later states, from data obtained from students at a college at Canyon, that "the area in Texas alone, mapped from data collected from the student body of the West Texas State Teachers' College, includes 86 of the 252 counties of Texas." The counties in the State affected were not stated. In a recent article on the geographical distribution of mottled enamel, McKay (*9*) states with respect to Texas: "The distribution over Texas is so widespread that no detailed cataloging of the communities is here possible, but reports indicate that the northwest portion known as the Panhandle constitutes by far the most extensive afflicted area in the country, and involves more people. A definite deep-well association has been reliably established. There are afflicted localities in other parts of the State."

In view of the widespread distribution of mottled enamel in Texas, two forms of survey were followed. As was the custom in each State, a questionnaire was sent to each component or district society in the State. In addition, 59 counties of the Panhandle and west Texas were made the object of a more intensive and complete survey.

Excluding the counties in the Panhandle and west Texas, the only area reported in the replies received from the various component societies is one at Taylor, in Williamson County, where it is estimated that there are 1,000 cases, and where the Austin Dental Society states that the condition is associated with the use of drinking water obtained from artesian wells. This report has likewise been confirmed by individual reports from dentists practicing in Taylor.

The Panhandle and West Texas

In order to obtain as much information as possible regarding mottled enamel in the Panhandle, a survey by counties was undertaken. Fifty-nine counties are included in this area. In counties where, according to Polk's Dental Register, there was no dentist practicing or where after a reasonable time no reply was received from the dentist practicing there, a questionnaire was sent to the county health officer. Often there were counties where no dentist was listed, and in these cases the sole report was from the county health officer. There were 13 such counties. In three counties, Borden, Yoakum, and Loving, it was impossible to find either a physician or a dentist to whom an inquiry could be addressed.

One hundred and twenty-nine questionnaires were sent into this area, 127 to individuals and 1 each to the Amarillo and Panhandle Dental Societies, respectively. Replies were received from 86 individuals and 2 societies, while 4 more were returned marked "unclaimed," "deceased," etc. The majority of the answers included valuable supplemental data. Excluding the three counties previously referred to where it was impossible to address an inquiry, we find that there are only 4 counties to which questionnaires were addressed and from which we have no report, or, in other words, we have 1 or more reports from 52 of the 59 counties being studied. These 52 counties are covered by 86 individual reports and 2 reports from societies.

Information in detail from counties of the Panhandle and west Texas follows:

Andrews County: No record of a dentist practicing in this county. The county health officer states that about 50 percent of the population is affected and that it is localized in the extreme western part of the county associated with deep-well water.

Armstrong County: The only report from this county is that from the county health officer. He reports mottled enamel locally but states that the incidence is only about 5 to 10 percent. He further adds that the water supply is obtained from deep wells 140 to 240 feet in depth.

Bailey County: A report from the county health officer, A. R. Matthews, and jointly signed by A. E. Lewis, D.D.S. states that practically all native-born children are affected. They state that the water is obtained from shallow wells.

Borden County: It was not possible to obtain the name of either a physician or dentist practicing in this county.

Brisco County: Dr. Ben R. Ezzell, a practicing dentist of Quitague, sends a complete report concerning mottled enamel in this county. He calls attention to the fact that Quitague is located just below the Cap Rock, at an altitude of 2,600 feet. About 10 miles to the west is the top of Cap Rock, where the Great Plains begin at an altitude of 3,100 feet. Dr. Ezzell states that mottled enamel occurs with much greater frequency on the Plains than in the valley. He also states that 3 years ago he examined over 900 school children in this county and found mottled enamel in the mouths of 10 percent of the children living in the valley and 25 percent of the children on the Plains. He states that the South Plains is underlaid with what is known as "the South Plains sheet water," which is tapped at from 20 to 40 feet anywhere. This South

Plains water is soft, while the water in the valley is hard. Dr. Ezzell adds that most of the mottled enamel occurs in the western part of the county around Silverton.

Carson County: Dr. O. York, the county health officer, reports only a few cases in this county and thinks they are confined to the shallow-water belt. The incidence is so low that the county is considered negative.

Castro County: Dr. Mayes Miller, county health officer, states that 85 percent of the native-born children of this county are affected. He also notes that mottled enamel is apparently evenly distributed throughout the county and that the water is obtained from wells 100 to 150 feet deep.

Childress County: Although occasional cases are sometimes seen, replies from three different dentists all indicate that this county is essentially negative.

Cochran County: There is neither a dentist nor a county health officer listed in this county. A questionnaire was addressed to the one physician practicing here but no reply has been received.

Collingsworth County: The report from Dr. W. R. Orr, a dentist who has practiced there for 21 years, indicates that the county is negative.

Cottle County: Dr. W. T. Payne reports that he recently examined the mouths of 300 school children between the ages of 6 and 12. No native-born children of this county showed mottled enamel. The county is listed as negative.

Crosby County: Reports have been received from Dr. F. A. Greene of Crosbyton, and Dr. H. F. Schwab of Ralls. They estimate an incidence of 60 and 50 percent, respectively, and state that the condition is general throughout the county. They add further that the drinking water is obtained from wells 100 to 300 feet deep.

Dallam County: Four replies have been received from this county, three from Dalhart and one from Texline. They indicate that what cases they see are from other areas, namely, Amarillo, Plainview, Lamesa, and the range country to the south. The county is listed as negative.

Dawson County: Five questionnaires were sent to dentists in this county and five replies were received. All reports indicate that mottled enamel is endemic. Dr. R. F. Nix, of Lamesa, for instance, stated that 95 percent of native-born children are affected. All five agree that shallow wells are associated with the dystrophy.

Deaf Smith County: Six questionnaires were sent into this county. Dr. G. W. Heard, a dentist, reports a low incidence, with considerable brown stain. Dr. G. F. Le Grand, a physician, of Hereford, roughly estimates an incidence of 5 percent but states that, due to the shifting population, it is difficult to estimate. He states that the drinking water is obtained from wells which vary in depth from 50 to 300 feet. Although the data with respect to this county is not as complete as might be desired, it is being listed as an endemic area, since all surrounding counties are endemic areas and the "spot mapping" of the Panhandle as a whole marks it as being in the mottled enamel zone.

Dickens County: Two replies have been received, which indicate a low incidence of mottled enamel. It is apparently more marked in that part of the county around McAdoo, according to a reported survey of school children.

Donley County: This county is apparently negative for mottled enamel.

Ector County: The county health officer, Dr. Emmet V. Headlee, reports this county as negative. No reply was received from the only dentist practicing in the county.

Floyd County: Three replies from this county all indicate that mottled enamel is endemic among the native-born children. Water from wells 100 to 200 feet deep is apparently associated with the dystrophy.

Fisher County: This county is reported as negative.

Gaines County: The county health officer, Dr. A. L. Bradford, reports an incidence of about 75 percent and notes an association with wells about 100 feet in depth.

Garza County: The county health officer, Dr. D. C. Williams, conservatively estimates that 50 percent are affected and notes an association with deep-well water.

Gray County: This county is reported as negative.

Hale County: Three replies were received from this county. Dr. P. E. Berndt estimates that 40 to 50 percent are affected, while both Dr. C. D. Wofford and Dr. W. J. Lloyd estimate that at least 90 percent are affected. They report both shallow and deep wells, principally shallow wells.

Hall County: Two replies indicate that this county is apparently negative.

Hansford County: A joint report from Dr. G. P. Gibner, county health officer, and Dr. F. J. Daily, a dentist, indicates that this county is negative

Hartley County: No replies received; two questionnaires sent.

Hemphill County: Dr. E. H. Snyder, county health officer, reports this county negative for mottled enamel with respect to native-born children.

Hockley County: Dr. Ray Ross states that the condition is general throughout the county, and adds that his investigation have shown that mottled enamel is always associated in this county with the use of shallow wells. Those using deep wells or artesians are apparently immune.

Howard County: Four replies from dentists at Big Spring mark this county as questionable. A few cases are reported, but from the country districts. These cases are apparently a mild, white, opaque type, with little or no brown stain such as is seen farther to the northwest. D. E. O. Ellington and Dr. W. B Hardy report a few cases in the northeast section of the county. This checks up with the fact that a few cases are reported in the southwestern section of Scurry County. Shallow wells are apparently used for drinking water.

Hutchinson County: Dr. W. H. Beckley, of Borger, states that 50 percent of the native-born children are affected in this county.

Jones County: Dr. F. C. Prichard of Anson, reports this county as negative for mottled enamel.

Kent County: Dr. J. H. Fowler, the county health officer, reports this county negative.

King County: No report.

Lamb County: Dr. C. C. Clements reports that nearly every child born at Littlefield has mottled enamel.

Lipscomb County: The county health officer reports the county negative.

Loving County: It was impossible to obtain the name of either a physician or dentist practicing in this county.

Lubbock County: Five replies were received from the city of Lubbock and two from Slaton. The consensus of opinion as conservatively expressed is that at least 50 percent of the native-born children are affected. Most of the cases are apparently associated with the use of shallow wells, but there are some deep wells in the county.

Lynn County: Dr. J. R. Singleton estimates that approximately 60 percent are affected and associates it with the use of shallow wells, which he states are about 110 feet deep.

Martin County: The county health officer reports a very low incidence of mottled enamel in this county. There is no dentist listed as practicing in this county. In as much as dentists in Dawson County to the north and Howard County to the east have both called attention to mottled enamel in Martin County, it is listed as an endemic area.

Midland County: Drs. L. B. Pemberton, K. P. Campbell, and D. K. Ratliff, all of Midland, report a fairly high incidence of mottled enamel in Midland County.

Mitchell County: This county is considered questionable. Dr. B. J. Dunlaney, of Colorado, reports an area about 12 miles to the northeast, but does not know the degree of involvement or the number affected. He states that shallow wells are used.

Moore County: Reports from two physicians and one dentist indicate that this county is negative for mottled enamel among the native-born children.

Motley County: Dr. E. W. McKenzie reports this county negative although he states that the condition is quite common in the counties to the west in the Plains country.

Nolan County: Three replies indicate that this county is negative for mottled enamel.

Ochiltree County: This county is apparently negative.

Oldham County: Dr. F. A. Collins, the county health officer, reports mottled enamel in this county. There are no dentists listed as practicing in the county.

Parmer County: Dr. J. M. McCuan, the county health officer, reports mottled enamel in this county. There are no dentists listed as practicing in the county.

Potter County: The secretaries of the Amarillo and the Panhandle Dental Societies, reporting from Amarillo, state that a high incidence of mottled enamel exists locally. Apparently deep wells are the sources of water supply here.

Randall County: A report from Dr. S. L. Ingham, a dentist, and Dr. C. A. Pierle, head of the department of chemistry, West Texas State Teachers' College, indicates a high incidence, approximately 75 percent, in this county. They state that both artesian and shallow wells are used.

Roberts County: The county health officer reports the county negative for mottled enamel.

Scurry County: Dr. Sed A. Harris reports only a few cases, those in the southwestern part of the county. It is interesting that a few cases were also reported in the northeastern part of Howard County. Scurry County is listed as questionable.

Sherman County: Apparently negative.

Stonewall County: Apparently negative.

Swisher County: Dr. R. L. Massey reports mottled enamel in this county and states that water is obtained from shallow wells.

Terry County: The county health officer, Dr. T. L. Treadway, reports mottled enamel in this county. No further data.

Wheeler County: Apparently negative.

Wrinkler County: No reply was received from the inquiry addressed to the county health officer. There is no dentist listed as practicing in the county.

Yoakum County: It was not possible to obtain the name of either a physician or a dentist practicing in this county.

Utah

Beaver County: Dr. Edgar A. Petty, of Beaver, calls attention to the near-by communities of Greenville and Adamsville, where, he states, 700 to 1,000 are affected. He estimates the incidence at 90 percent for both towns and notes that drinking water is obtained from artesian wells and deep springs.

Washington County: Both Dr. E. H. Conger and Dr. W. C. Cox, of St. George, report mottled enamel in St. George, but note that it is not nearly as severe as formerly. They state that about 20 years ago the water supply was changed. Formerly the entire municipal water supply was obtained from springs and mottled enamel in St. George was very severe. It is not very clear in either report whether the new water supply from Pine Valley mountains is now used exclusively in the municipal supply for domestic purposes, or whether this mountain water has been merely added to the local spring supply. One report states that the St. George spring water is still being used for irrigation of truck gardens and vegetable plots. Both agree that there has been a marked decrease in the incidence of mottled enamel since the change in the water supply.

Virginia

Areas of mottled enamel at Franklin and Courtland, Southhampton County, were reported by McKay (*3*) in 1916. In 1919, he (*13*) reported the results of a survey at Franklin. In 1931, the writer examined 222 school children at the Courtland school. These children were from Courtland and the surrounding rural districts within a radius of 6 or 7 miles. In this group were 29 children who had lived in Courtland and used artesian water exclusively from birth. Twenty-eight, or 96 percent, showed definite signs of mottled enamel. The one remaining case, listed as negative, was in a 6-year old child whose erupted permanent teeth were limited to the lower central incisors. The water supply in Courtland is obtained from individual flowing artesian wells. Reports from the State department of health indicate that mottled enamel is present at Smithfield (confirmed by Dr. W. C. Ames, a dentist of Smithfield). Rescue, Battery Park, and Carrollton in Isle of Wight County and Chuchatuck in Nansemond County. Dr. W. L. Smoot, of Williamsburg, writes that practically all native-born children of Jamestown Island are affected. He states that artesian water is used for drinking purposes.

Questionable Areas

Questionable areas include parts of Howard, Mitchell, and Scurry Counties, Tex.; Dona Ana County, N.M.; and the "South Pass and Atlantic City" district in the southern part of Fremont County, Wyo.

SUMMARY

There are 97 localities which have been referred to in the literature as areas where mottled enamel is definitely demonstrable or areas reported by questionnaires and subsequently confirmed by survey of the locality.

There are, in addition, 28 areas referred to in the literature as mottled-enamel areas. No confirmatory surveys are reported, but little doubt exists as to the production of mottled enamel in these regions. They are, in the main, in the Panhandle and west Texas.

There are 70 areas which have been reported by questionnaires but which have not as yet been confirmed by extensive surveys.

There are 5 areas reported by questionnaires which are held as questionable pending receipt of further data.

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CLASSIFICATION OF MOTTLED ENAMEL DIAGNOSIS

By H. TRENDLEY DEAN

During the past few years, considerable interest has been evinced in mottled enamel. Its world-wide distribution makes it a problem of more than national interest. In addition to its widespread distribution in the United States (*1*), endemic mottled enamel has been reported in England (*2*), Italy (*3*), North Africa (*4*), Argentine (*5*), Japan (*6*) and China (*7*) and in numerous other places (*8*).

Surveys that I have made in areas of varying degrees of severity have clearly demonstrated the need for a method of clinically classifying the degree of involvement. Not only is there a qualitative variation among persons using a common water supply, but there is also a marked quantitative difference in incidence among children in different endemic areas. While it is true that the degree of involvement may vary somewhat among different teeth in the same person, it is felt that each person examined should be classified in a manner that would designate in a general way the degree of involvement as found in that person.

In a number of surveys (*1*), I have used the following classification: normal, questionable, very mild, mild, moderate, moderately severe, and severe. Little difficulty is encountered by the general practitioner in the diagnosis of those types of mottled enamel ranging from "mild" to "severe." To the experienced investigator, "very mild" presents separate and distinct characteristics. The classification of "questionable" is often a baffling problem. There are areas where the causative factor in mottling of the enamel is apparently just between the maximum harmless amount and the minimum amount capable of producing the milder forms of mottling. In such areas, occasional minute flecks and small white spots on the enamel often show in an unusually high percentage of children.

Slight aberrations in the translucency of normal enamel have been referred to by various dental pathologists. Black (*9*) states that the smaller white or ashy gray spots which occur on the enamel of teeth, otherwise normal in color and form, are due to the absence of the cementing substance between the rods, which are normal, and that this mild form of dystrophy generally ends in a line following the incremental lines of enamel formation; i.e., the lines of Retzius. He states that these smaller white spots are not infrequently seen in any large school clinic. Later, McKay (*10*), in referring to Black's clas-

Fig. 1

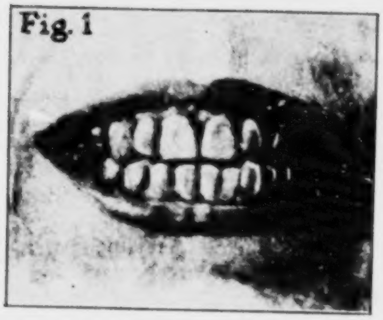

FIGURE 1.—*Normal condition. The apparent white spots depict "high lights" and are not mottled enamel.*

Fig. 2

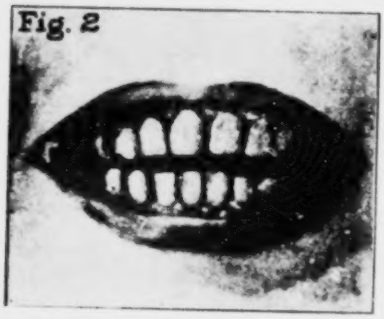

FIGURE 2.—*Questionable condition.*

sification of dystrophy, states that white spots, white enamel and mottled enamel are fundamentally the same lesion.

Bunting (*11*) writes that small white opaque areas in the enamel are distinctly hypoplastic in structure; that the rods may be fairly well formed, but the interprismatic cement substance is absent or deficient, the histologic picture being similar to that of incipient caries. Williams (*12*) states that white spots may be congenital or acquired and, in both instances, the condition and appearance are similar. In his opinion, the peculiar opaque appearance is due to the absence of cementing substance which causes diffraction of light. He suggests that the acquired spots are the result of the presence of aciduric organisms operating under a bacterial plaque, while in the congenital group he places mottled enamel. Kronfeld (*13*) refers to the congenital white spots sometimes observed on the labial surfaces of the incisors as indicative of preliminary or abortive stages of hypoplastic enamel, while, with respect to early caries on the smooth surfaces of enamel, clinically known as "white spots" or "brown spots," his inferences are apparently similar to those of Williams and also of Bunting. Kronfeld also notes that such areas of superficial decalcification may develop into true carious defects, or they may remain stationary for a long time, even for a lifetime. Ainsworth (*2*), in his recent report on the histopathology of mottled enamel, states that the microscopic appearances are strikingly like those of early decalcification of the enamel from caries. This investigator likewise notes that isolated opaque patches are not infrequently seen in persons whose teeth are otherwise normal and whose habitat has been nonendemic areas. From the foregoing, it is evident that the classification of "questionable" cases, namely, as to whether they are the earliest signs of mottled enamel or of other abnormalities, may present difficulties. The method of utilizing this classification with particular reference to mottled enamel will be described in detail later.

Fig. 3

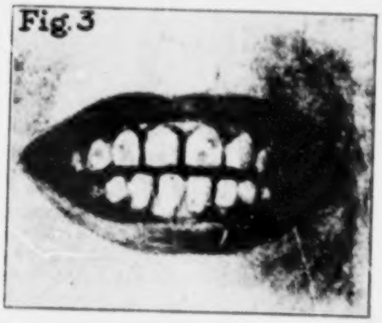

FIGURE 3.—*Very mild case of mottling.*

In mottled enamel surveys, I classify each person immediately after the clinical examination. The presence or absence of mottled enamel is noted on the special examination chart in accordance with one of the seven classifications previously referred to, ranging from "normal" to "severe." The basis for each classification is as follows:

NORMAL (fig. 1)

The enamel presents the usual translucent semivitriform type of structure. The surface is smooth and glossy and usually of a pale creamy white color. In addition to those showing normal calcification, for purposes of classification, there is also included under this heading all persons showing hypoplasia other than mottling of the enamel. Such hypoplasia of the enamel is, in the main, hutchinsonianism and that seen in association with exanthematous disease and nutritional disturbances occurring during the period of the enamel development of the permanent teeth, as described by Bunting (*11*). If an examination of a person reveals the presence of one of the previously mentioned types of hypoplasia and mottling of the enamel, the examination is recorded solely on the basis of the mottled enamel present and is listed under its proper classification.

Fig. 4

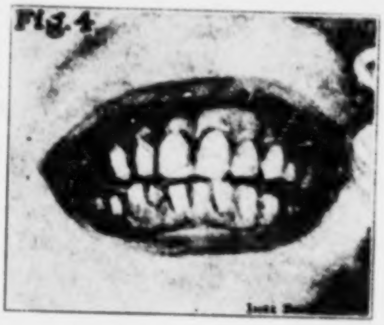

FIGURE 4.—*Mild mottling.*

QUESTIONABLE (fig. 2)

In areas of relatively high endemicity (over 75 percent), there are occasional cases which even the experienced investigator hesitates to classify as either apparently normal or very mild. Such cases are listed as questionable. The common use of this classification occurs in areas where an unusually high percentage of children show slight aberrations in the translucency of normal enamel, ranging from a few white flecks to occasional white spots, 1 to 2 mm. in diameter. In such areas, the following method of differential diagnosis is followed: First, no attempt is made to diagnose these small white spots or minute white fleckings as the earliest signs of mottling of the enamel by examination of the person *per se*. Recourse is always made to group study. For example, a group of 25 children whose history shows a continuous use of a common water supply from birth are examined. Another group of equal number and of similar economic or living conditions are selected from a nearby community as a "control." This second group must of course show a history of also using a common water supply exclusively from birth and from a source different from that of the first group. It is of course desirable to have larger numbers in each group, but more can be learned from a small group with the same water supply from birth than from a much larger group wherein different water supplies have been used during the period of calcification of the permanent teeth.

On examination, the first group may, for instance, show 14 children with apparently normal enamel, 8 with occasional white flecks or small white opaque spots on the enamel surface, 2 with a very mild mottled enamel and 1 with a mild mottled enamel. The "control" group, on

Fig. 5

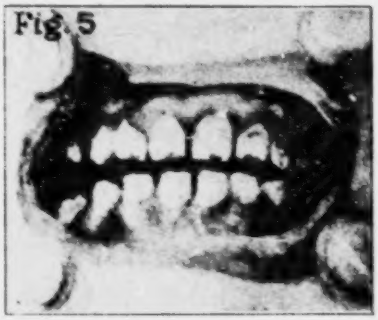

FIGURE 5.—*Moderate (white opaque) mottling.*

the other hand, yields 22 children with apparently normal calcification and 3 with white flecking or white spots. The eight questionable cases in the first group are naturally suggestive, as three additional children with the same water history, but who were probably more susceptible than the others, show definite signs of mottled enamel. The few questionable cases in the control group are probably no greater in number than might be expected in an examination of an average school group. Thus, in areas of questionable endemicity, this classification becomes a valuable adjunct. Should an unusually large number of cases be classified as "questionable" when, at the same time, a smaller percentage is likewise showing the milder forms of mottling of the enamel, we are justified in assuming that we are dealing with what is probably a "borderline" area, an area where the causative factor in the mottling is present in the water supply quantitatively somewhere between the maximum harmless amount and the minimum amount capable of producing the very mild and the mild type of mottled enamel in from 40 to 50 percent of the children who have used the water in question exclusively from birth.

Fig. 7

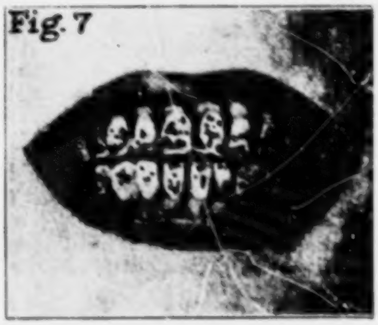

FIGURE 7.—*Moderately severe mottling, with discrete pitting.*

Fig. 6

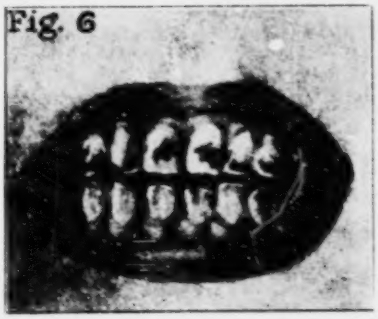

FIGURE 6.—*Moderate mottling (brown stain).*

VERY MILD (fig. 3)

Small opaque paper white areas are scattered irregularly or streaked over the tooth surface. It is principally observed on the labial and buccal surfaces, and involves less than 25 percent of the tooth surfaces of the particular teeth affected. Small pitted white areas are frequently found on the summit of the cusps. No brown stain is present in the mottled enamel of this classification.

In areas of high endemicity, mottled enamel is occasionally observed on the deciduous molars, generally the second deciduous molar. In my experience, mottling of the enamel on deciduous teeth is almost invariably of the very mild type, even though the permanent teeth in the same individual may show a severe mottling.

MILD (fig. 4)

The white, opaque areas on the surfaces of the teeth involve at least half of the tooth surface. The surfaces of molars, bicuspids, and cuspids subject to attrition show thin white layers worn off and the bluish shades of underlying normal enamel. Faint brown stains are sometimes apparent, generally on the upper incisors.

Fig. 8

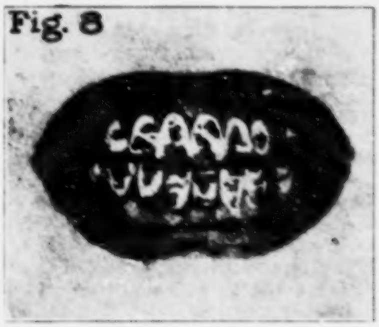

FIGURE 8.—*Severe mottling, with confluent pitting.*

MODERATE (figs. 5–6)

No change is observed in the form of the tooth, but generally all of the tooth surfaces are involved. Surfaces subject to attrition are definitely marked. Minute pitting is often present, generally on the labial and buccal surfaces. Brown stain is frequently a disfiguring complication. It must be remembered that the incidence of brown stain varies greatly in different endemic areas, and many cases of white opaque mottled enamel, without brown stain, are classified as "moderate" and listed in this category.

Fig. 9

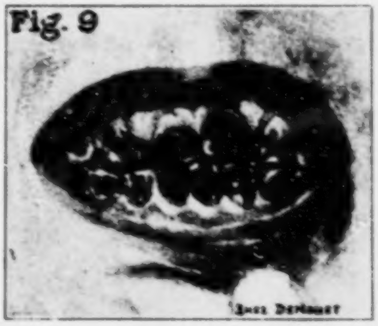

FIGURE 9.—*Severe mottling (brown stain).*

MODERATELY SEVERE (fig. 7)

Macroscopically, a greater depth of enamel appears to be involved. A smoky white appearance is often noted. Pitting is more frequent and generally observed on all the tooth surfaces. Brown stain, if present, is generally deeper in hue and involves more of the affected tooth surfaces.

SEVERE (figs. 8–9)

The hypoplasia is so marked that the form of the teeth is at times affected, the condition often being manifest in older children as a mild pathologic incisal-occlusal abrasion. The pits are deeper and often confluent. Stains are widespread and range from a chocolate brown to almost black in some cases. Under this classification, I include the type of case which McKay (*14*) has referred to as the "corrosion" type of mottled enamel.

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MOTTLED ENAMEL IN TEXAS

By H. Trendley Dean, R. M. Dixon, and Chester Cohen

INTRODUCTION

Since 1916 there have been occasional references (*1*), (*2*), (*3*), (*4*), in the literature inviting attention to the presence of mottled enamel in west Texas. In 1932 (*5*) a detailed questionnaire survey by the United States Public Health Service indicated that the Panhandle-west Texas region was probably the largest mottled enamel area in the United States with more people affected. This report showed that there were at least 26 west Texas counties in which mottled enamel was endemic and that such large centers of population as the cities of Amarillo, Lubbock, and Plainview were seriously affected. In addition, the possibilities of other affected areas in Texas became evident when mottled enamel was reported as endemic at Taylor, in Williamson County. Lemmon (*6*), a pediatrician, has recently called to the attention of the Texas medical profession the relationship between mottled enamel and child hygiene and nutrition.

METHOD OF SURVEY

This survey was a cooperative study made by the United States Public Health Service and the Texas State Department of Health during November and the early part of December 1934. Each of the communities hereinafter referred to was visited, and subsequently, with the cooperation of the local superintendent of education, school children, generally of the fourth, fifth, and sixth grades, were examined. A total of 66 cities, towns, or rural communities in 44 counties was visited and 3.723 school children were examined. The purpose of the survey was to obtain general information relative to the extent of the affected territory and a rough index of the degree of severity of the mottled enamel being produced.

Upon visiting a classroom, the purpose of the survey was first explained, and those children who had lived in the community continuously since birth and who had always used the city water for domestic purposes (cooking and drinking) were assembled in a separate group. This group was further questioned to determine whether there had been any breaks in the continuity of their residence and water consumption. Under good illumination each child was examined by one of us (H. T. D.) and the presence or absence of mottled enamel recorded. The degree of severity was noted in accordance with a standard of classification previously described (7). In many instances the children with variable residences and water histories were likewise examined under the same conditions.

The basis upon which the various degrees of mottle enamel were classified is briefly, as follows:

NORMAL (fig. 1)

The enamel presents the usual translucent semivitriform type of structure. The surface is smooth, glossy, and usually of a pale, creamy white color. In addition to those teeth showing normal calcification, for purposes of mottled enamel classification there is also included under this heading all individuals with permanent teeth showing hypoplasias *other* than mottled enamel. Such hypoplasias of the enamel are, in the main, those characteristic of Hutchinson's teeth and the hypoplasias concomitant with the exanthematous diseases and nutritional disturbances during the period of the enamel development of the permanent teeth. If an examination of a person reveals the presence of one of the previously mentioned hypoplasias *and* mottled enamel, the examination is recorded solely on the basis of the mottled enamel present and is listed under its proper mottled enamel classification.

QUESTIONABLE (fig. 2)

In areas of relatively high endemicity, over 75 percent, there are at times cases which the experienced investigator occasionally hesitates to classify either as apparently normal or very mild. Such cases are listed as questionable. In studying a "border line" area, or a community where the causative factor of mottled enamel is present in the water supply quantitatively somewhere between the maximum harmless amount and the minimum capable of producing the "very mild" and "mild" type of mottled enamel in 35 percent or more of the children who have used the particular water exclusively from birth, this classification is frequently needed. In such areas there is generally a higher percentage of individuals classed as normal than the combined group of "very mild" and "mild." There is, however, always a certain percentage of those individuals with comparable histories, that discloses slight aberrations in the translucency of normal enamel ranging from a few white flecks to occasional white spots. Furthermore, in some instances, thin, irregular, white, opaque streaks, or veining, are noted on the incisal third of the superior incisors. In other cases the tip of the summit of the bicuspids shows an unusual white opacity two or three millimeters in extent, the remainder of the tooth being apparently normal. As such cases are not sufficiently developed to be classed as "very mild", and are definitely not "normal", they are listed as questionable.

Very Mild (fig. 3)

Small, opaque, paper-white areas are scattered irregularly or streaked over the tooth surface. This mottling is principally observed on the labial and buccal surfaces and involves up to 25 percent of the tooth surface of the particular teeth affected. Small, pitted, white areas are frequently found on the summit of the cusps. Brown stain is rarely observed in the mottled enamel of this classification and, if present at all, is so faint as to be almost indistinct.

In areas of high endemicity, mottled enamel is not infrequently observed on the deciduous molars and occasionally the deciduous cuspids. Mottled enamel in deciduous teeth is generally of the very mild type, even though the permanent teeth in the same individual may show moderate to severe mottling.

Mild (fig. 4)

The white opaque areas in the enamel of the teeth involve at least half of the tooth surface. The surfaces of molars, bicuspids, and cuspids subject to attrition show thin white layers worn off and the bluish shades of underlying normal enamel. Light brown stains are sometimes apparent, generally on the superior incisors.

Moderate (figs. 5 and 6)

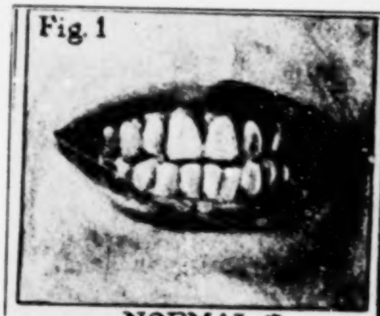
No change is observed in the form of the tooth, but generally all tooth surfaces are involved. Surfaces subject to attrition are definitely marked. Minute pitting is often present, generally on the labial and buccal surfaces. Brown stain is frequently a disfiguring complication. For the most part the stain ranges from tan to chocolate in color and not infrequently involves as much as half of the labial surface. It must be remembered, however, that the incidence of brown stain varies greatly in different endemic areas and many cases of white opaque mottled enamel, without brown stain, are classified as "moderate" and listed in this category.

Moderately Severe (fig. 7)

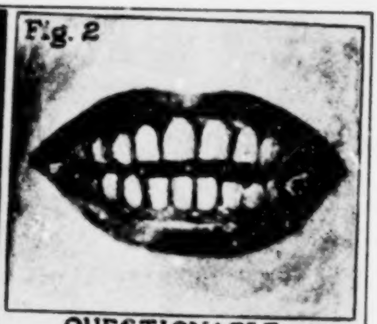
Macroscopically a greater depth of enamel appears to be involved. A smoky white appearance is often noted. Pitting is more frequent and generally observed on all tooth surfaces. The pits are descrete and may be 1 to 2 millimeters in diameter. Brown stain, if present, is generally deeper in hue and involves more of the tooth surface. The diagnostic sign of this classification is, however, the discrete pitting.

Severe (figs. 8 and 9)

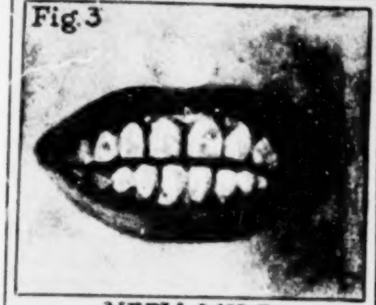
The hypoplasia is so marked that the form of the teeth is at times affected; the older children often present a mild incisal-occlusal pathological abrasion. The pits are deep and very often confluent. As a result of confluent pitting, which is the diagnostic sign of this classification, the outer surface of the enamel is lost in places and the tooth often presents a corroded-like appearance. Stains are widespread and range in color from chocolate brown to almost black.



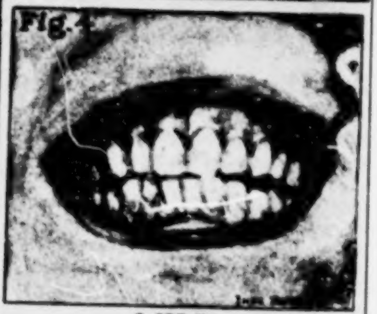
NORMAL *



QUESTIONABLE



VERY MILD



MILD

* THE APPARENT WHITE SPOTS ON THE LABIAL AND BUCCAL SURFACES IN THIS REPRODUCTION (NORMAL) DEPICT "HIGH LIGHTS" AND ARE NOT MOTTLED ENAMEL.

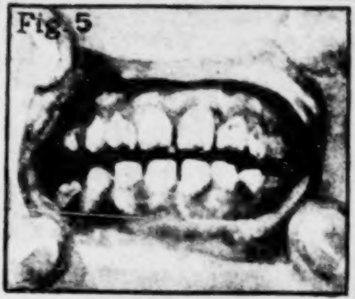


Fig. 5

MODERATE
White opaque

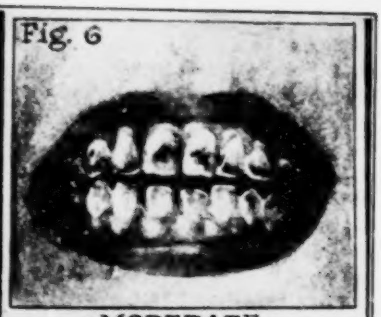


Fig. 6

MODERATE
Brown stain

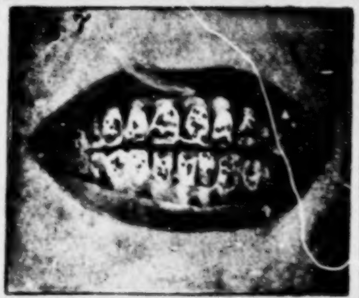


Fig. 7

MODERATELY SEVERE
Discrete pitting

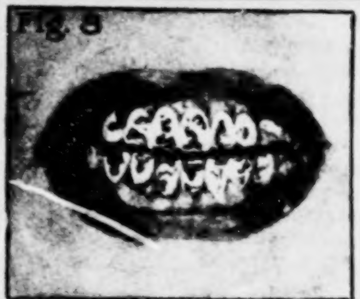


Fig. 8

SEVERE
Confluent pitting

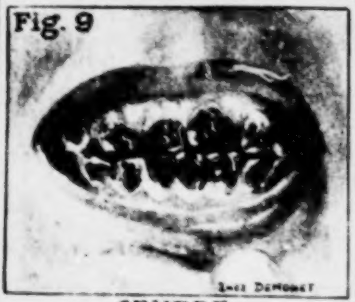


Fig. 9

SEVERE
Brown stain

MOTTLED ENAMEL INDEX OF A COMMUNITY

The various degrees of mottled enamel severity having been defined, the application of this classification to the determination of a mottled enamel index of a community is necessary for epidemiological purposes and subsequent correlation with chemical and other studies.

Accordingly the following indexes have been arbitrarily defined in terms of the degree of severity of mottled enamel observed clinically:

Negative: When less than 10 percent of the children show "very mild" or more severe types of mottled enamel.

Border Line: When 10 percent or more, but less than 35 percent, show "very mild" mottled enamel or worse.

Slight: 35 percent or more show "very mild" or worse, but less than 50 percent are mild or worse, and less than 35 percent "moderate" or worse.

Medium: 50 percent or more are mild or worse, but less than 35 percent are "moderate" or worse.

Rather Marked: 35 percent or more, but less than 50 percent are "moderate" or worse, but less than 35 percent are "moderately severe" or worse.

Marked: 50 percent or more are "moderate" or worse, but less than 35 percent are "moderately severe" or worse.

Very Marked: 35 percent or more are classified as "moderately severe" or worse.

All children included in a group utilized in the determination of a mottled enamel index of a community refer to children whose time of risk of exposure had been constant, meaning that the children were born in the community, had lived there all their lives (short vacations totaling less than 30 days in 1 calendar year excepted), and had always used the municipal or common water supply for cooking and drinking purposes. In certain west Texas communities the mottled enamel index could be determined only tentatively at this time. The reason for a tentative index will be made apparent in the section dealing with factor of population influx.

FACTOR OF POPULATION INDEX

The factor of population changes and its relation to changes of water supply are obviously of paramount importance in mottled enamel investigation. The pertinent facts concerning population movements have a direct bearing on the west Texas survey. There has been a rapid growth and development of west Texas during the period between 1920 and 1930. The marked migration into west Texas during this decade is well illustrated by an examination of the reports of the Bureau of the Census (*8*). The percentage increase in population between 1920 and 1930 for the State of Texas was 24.9, while the population of the 37 west Texas counties covered by this report increased from 138,851 in 1920 to 379,881 in 1930, or 173.6 percent.

As a result of the unusual increase in population in west Texas during the period between 1920 and 1930, a large number of children disclosed histories of residence in nonendemic and endemic areas, or of having lived continuously since birth in a community where the municipal water supply had been installed or changed during the life of the child. It was not infrequent to find that smaller cities or towns had installed municipal water only as late as 6 to 8 years ago; previous to that time the few inhabitants depended on windmill wells.

In all of such places the attempt was made to determine whether the municipal water supply was producing mottled enamel by an examination of those children in the fifth and sixth grades who had used the municipal water exclusively for at least the past 6 years. In such groups the examination was limited to the cuspids, bicuspids, and second molar teeth, and the presence or absence of mottled enamel recorded on the basis of these observations. Under such conditions the mottled enamel index given to such communities is necessarily tentative. Each community should be resurveyed 3 or 4 years hence to determine its mottled enamel index.

WATER SUPPLIES

In the west Texas phase of the survey, another of us (R. M. D.) obtained all

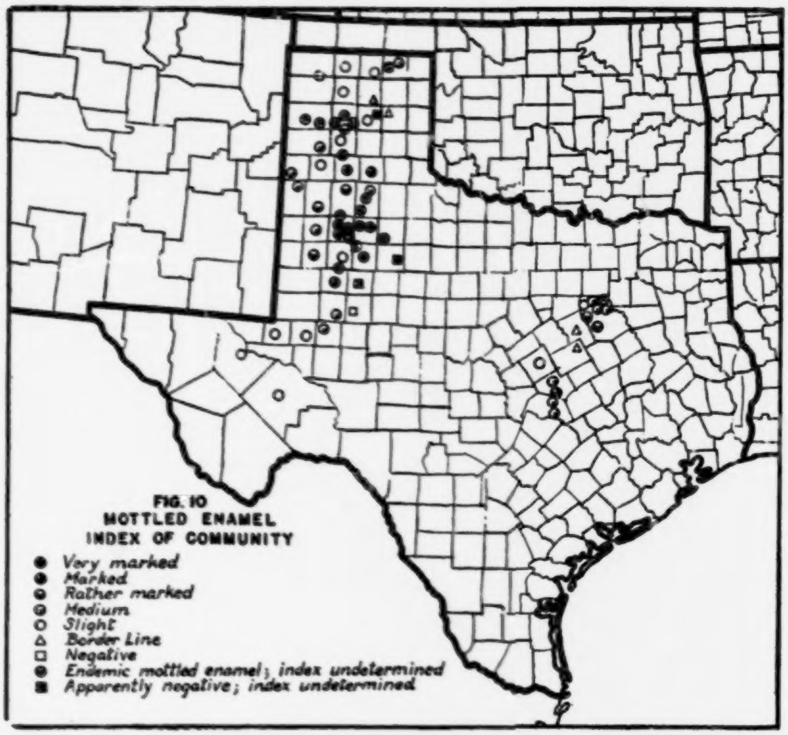

● Very marked
● Marked
◉ Rather marked
◉ Medium
○ Slight
△ Border Line
□ Negative
● Endemic mottled enamel; index undetermined
■ Apparently negative; index undetermined

FIG. 10 MOTTLED ENAMEL INDEX OF COMMUNITY

relative data available concerning the municipal supply from the local water superintendent, and collected one or more samples of the supply. When the municipal supply was a composite water from more than one stratum, two or more samples were collected whenever possible. These samples were forwarded to the Texas State Department of Health in whose laboratories the fluoride determinations are being made. The report of the chemical determination of these waters associated with endemic mottled enamel will be made the basis of a separate report. The information included in this report regarding municipal water supplies of the affected communities in the east central Texas area has been obtained by another of the authors (C. C.).

In west Texas there are apparently three strata of water-bearing sands, in general not widely separated in depth. Practically all wells in this region are drilled, and it is customary to refer to drilled wells obtaining water from the first stratum as "shallow," and from the second or third stratum as "deep." Consequently in one county the term "shallow" may be applied to a 300-foot drilled well because water from the second or third stratum is not obtained until a depth of 450 or 500 feet is reached, while in another county, the term "deep" well may be applied to a 125-foot well because the first stratum of water in that particular locality is reached at 80 feet.

SURVEY FINDINGS

The results of this survey are summarized as follows:

Table 1 details the mottled enamel findings and history of common water supplies in certain cities of the Panhandle, west Texas, and east central Texas. In these cities a sufficient number of children with a history of continuous residence and constant use of the city water were examined to warrant the development of an approximate or tentative mottled enamel index of the community. Ordinarily the mottled-enamel index of a community should not be determined unless the group examined consists of 25 or more children with a continuous residence since birth and a constant use of a common water supply. This minimum standard could not be adhered to in all instances in this survey owing to the factor of population changes or a smaller number of children available in the school showing a constant residence and water history. It should be noted that an "actual mottled enamel index" is not given a community unless all histories as given by the child, with respect to both residence and water supplies, are rechecked and confirmed by an interview with the child's parents. Figures 11 and 12 illustrate the percentage distribution of that part of the sample having continuous residence and constant use of a common water supply listed according to severity of affection, and they also show either the approximate or tentative mottled enamel index of the community.

In table 2 are listed four small communities possessing municipal water supplies, but where an insufficient number of examinations were made to permit the computation of a mottled-enamel index.

Table 3 summarizes mottled enamel findings in certain communities and rural districts of the Panhandle and west Texas where common water supplies are either not available or, in two instances, not used.

DISCUSSION

This survey presents definite evidence that the Panhandle-west Texas region constitutes the largest mottled-enamel area in the United States. There is no doubt that a detailed survey would disclose many additional smaller communities and rural districts where mottled enamel is endemic. Since this territory is generally affected through all graduations from a slight to a marked degree, the influence of the causative factor of mottled enamel is operative over a vast area, with the result that many thousands of the inhabitants are affected.

The area known as the "Llano Estacado" is apparently the most severely affected. From observations made during this survey, the region of the greatest severity centers in and around the city of Lubbock and extends in an easterly direction toward Spur and Post, northward toward Plainview and Amarillo, and southward toward Lamesa. The fact that such large cities as Amarillo, Lubbock, and Plainview are located in the region of the greatest severity makes this a serious problem of keen public-health interest. Although definite manifestations of endemic mottled enamel are readily demonstrable in communities located north of the Canadian River and south of the eastward prolongation of the southern boundary of the State of New Mexico (Edwards Plateau), the type of mottled enamel being developed in these two regions is markedly less severe, the community index generally being slight.

Examination of numerous children, who spent the first 5 or 6 years of their lives in eastern New Mexico, indicates

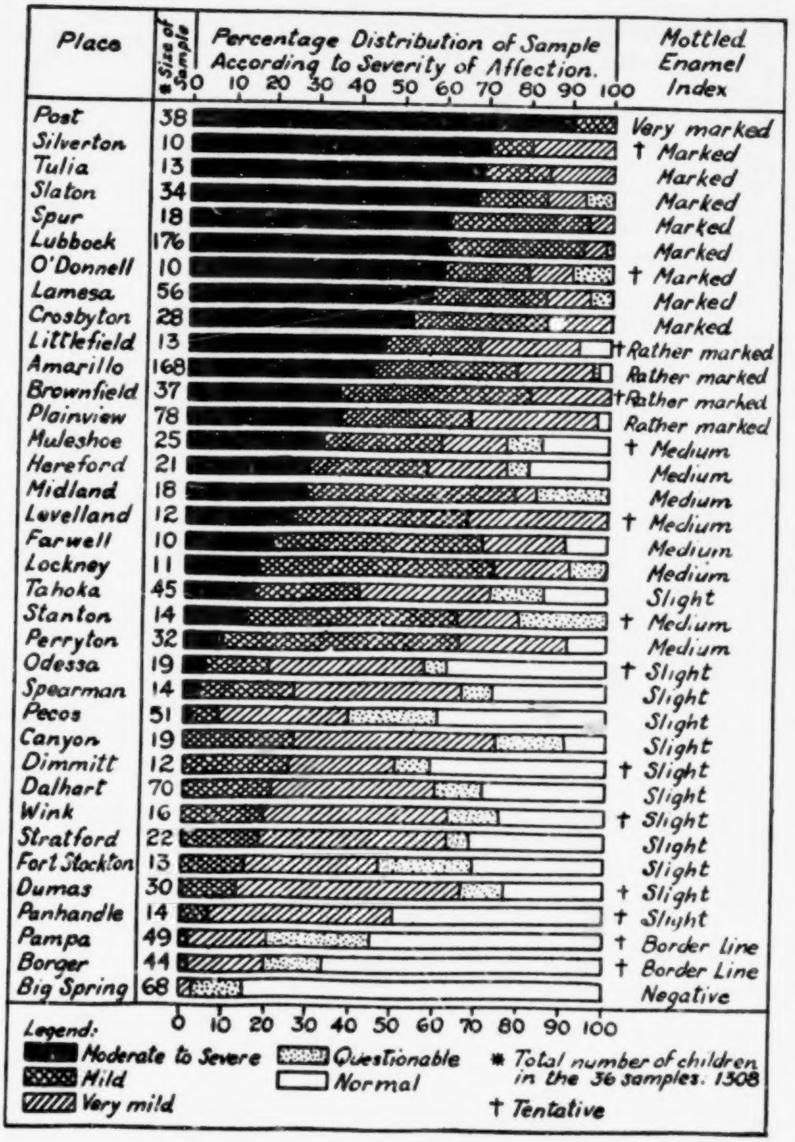


FIGURE 11.—*Severity of mottled enamel in children of certain selected places of the Panhandle and west Texas*

(Children in continuous residence and uninterrupted use of municipal water)

definitely that mottled enamel comparable to that found in west Texas is likewise being developed in eastern New Mexico.

The east central Texas area should be further studied and the boundaries of endemicity determined. Endemic mottled enamel has now been definitely demonstrated in numerous localities between Austin and Dallas. In certain communities, such as Bartlett, Italy, and Frost, a type of mottled enamel is being produced that is comparable in severity with some of the more seriously affected cities and towns of west Texas.

SUMMARY

(A) THE PANHANDLE AND WEST TEXAS

1. The Panhandle-west Texas region constitutes the largest mottled-enamel area in the United States. As a result of the unusual population influx between 1920 and 1930, the number of children affected has correspondingly increased.

2. Of 53 communities surveyed in 37 counties, only 6 could be classified as "negative" or "border line."

3. The fact that the municipal water supplies of such large cities as Amarillo, Lubbock, and Plainview contain the causative factor of mottled enamel in sufficient concentration to produce this hypoplasia in a high percentage of their children has developed an acute and urgent public health problem.

(B) EAST CENTRAL TEXAS

4. An endemic area of unknown size is reported in east central Texas between Austin and Dallas.

5. Of 13 communities surveyed, only 2 were classified as "border line" and none was classified as "negative."

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(*8*) Fifteenth Census of the United States; 1930, Vol. 1, Population. Number and Distribution of Inhabitants. Bureau of the Census, Washington, 1931.

TABLE 1.—*Summary of mottled enamel findings and history of water supply in certain cities of (1) Panhandle and west Texas and (2) east central Texas*

1. Panhandle and west Texas

City and population (census of 1930)	Total number of children examined	Children classified according to mottled enamel diagnosis														History of water supply	Remarks
		(A) Continuous residence with constant use of city water							(B) Changes in residence and/or water history								
		Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe	Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe		
Post (1,668)	38	0	0	0	4	19	13	2	(1)	(1)	(1)	(1)	(1)	(1)	(1)	Present supply in constant use since prior to 1922; obtained from 13 wells 85 feet to 100 feet deep; apparently in first stratum.	Sample represents all children in the fourth, fifth, and sixth grades who used city water continuously. Post is located east of the cap rock, but the wells from which the supply is obtained are 3 to 4½ miles west of Post, and on the cap rock.
Silverton (873)	45	0	0	2	1	6	1	0	7	1	8	8	7	4	0	Obtained from 120 feet (1924) and 150 feet (1928) wells in first stratum, similar to local windmill wells. City supply in general use last 6 years only.	Entire sixth grade examined; "B" also includes some children from immediate rural districts.
Tulia (2,202)	61	0	0	2	2	8	1	0	7	2	12	13	13	1	0	From North well 168 feet (1922) and South well 60 feet (1925). Apparently first stratum. Most of water supply at present from the South well.	Entire sixth grade examined; "B" also includes children from immediate rural district.
Slaton (3,876)	34	0	2	3	6	19	3	1	(1)	(1)	(1)	(1)	(1)	(1)	(1)	From 1922–24, shallow wells, first stratum. Since 1924, 3 wells, 125, 135, and 210 feet. First 2 take water from first and second strata; third well from all 3 strata.	Sample represents all children of fourth, fifth, and sixth grades who used city water continuously.
Spur (1,899)	24	0	0	1	6	11	0	0	4	0	0	1	1	0	0	Municipal water supply is obtained from 3 wells, each 44 feet deep. Spur is located east of the cap rock, but is apparently drawing its water from the cap rock aquifer.	Sample under "A" represents all children in fifth and sixth grades with constant history. Four normals under "B" used cistern water exclusively.
Lubbock (20,520)	176	0	2	11	54	77	31	1	(1)	(1)	(1)	(1)	(1)	(1)	(1)	Municipal water obtained from 8 drilled wells with standard steel casing the entire depth, and averaging 98 to 150 feet.	Sample represents all white children in fourth, fifth, and sixth grades of public schools whose histories indicated constant use of city water since birth.
O'Donnell (1,026)	27	0	1	1	2	6	0	0	2	1	6	5	2	1	0	Obtained from seven 80-foot uncased wells, taking water from first stratum. 5 were drilled in 1928 and 2 in 1932. No common water supply prior to 1928.	"B" includes children from immediate rural district and "A" sample, all children third, fourth, and fifth grades who used city water past 5 years.
Lamesa (3,528)	56	0	3	6	15	23	9	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	1921 to 1929 obtained from 3 wells, 90, 140, and 300 feet, respectively. Since 1929, 3 wells 140 feet, taking water from second stratum.	Sample represents all children in the third, fourth, and fifth grades who used city water continuously since birth.
Crosbyton (1,250)	28	0	0	4	9	14	1	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	From two 280-foot wells drilled in 1918 and 1930 and taking water from the second stratum.	Do.
Littlefield (3,218)	84	1	0	3	3	5	1	0	21	6	17	16	11	0	0	From four 120-foot wells drilled in 1926 and 1927, obtaining water from the first stratum. No common water supply prior 1926.	"A" sample represents all children in fifth and sixth grades who used city water continuously since installation.
Amarillo (43,132)	533	5	1	30	58	60	12	2	126	15	90	86	42	6	0	From 10 wells (1927) 180 feet deep and 5 (1931) 280-foot wells. Previous 1927 from thirty-five 250-foot wells located in various parts of the city.	Sample consists of entire fourth, fifth, and sixth grades in 3 white and 1 colored public schools.
Brownfield (1,907)	37	0	0	7	17	10	3	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	Since 1925 from 2 dug wells 103 and 117 feet, taking water from first stratum. Prior 1925 no municipal supply.	Sample represents all children in fourth, fifth, and sixth grades who used city water continuously since 1925.
Plainview (8,834)	78	2	0	24	24	26	2	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	From 3 wells 95, 175, and 275 feet. Prior to 1926 first 2 wells only were used; 1926–28 275-foot well used solely. Present supply composite of all 3 wells.	Sample represents children in fifth, sixth, seventh, and eighth grades, who used municipal water continuously since birth.
Muleshoe (779)	72	4	2	4	7	7	1	0	22	2	11	7	3	2	0	From 1 well (1927) 90 feet deep, obtaining water from first stratum and apparently comparable with many local windmill wells. No supply prior 1927.	"A" sample represents all children in fifth, sixth, and seventh grades, who used city water continuously for past 6 years.
Hereford (2,458)	92	4	1	4	6	4	2	0	11	8	15	16	18	3	0	From three 60-foot wells drilled in 1919, 1921, and 1925. Many individual windmill wells used prior to 1927 comparable in depth.	"B" sample includes many children using individual windmill wells, both city and immediate rural district.
Midland (5,484)	18	0	3	1	9	5	0	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	Since 1928 from 2 wells 130 feet; 1910–28, from wells approximately 90 feet.	Sample represents all children in fifth and sixth grades using city water continuously since birth.
Levelland (1,661)	77	0	0	4	5	3	0	0	16	8	15	13	12	1	0	From two 185-foot wells drilled 1928–29. Water obtained from second stratum; first stratum is cased off at 120 feet. No common water supply prior 1928.	"A" sample represents all children in fifth and sixth grades who used city water for past 6 years.
Farwell (647) [2]	52	1	0	2	5	2	0	0	20	2	13	5	2	0	0	Obtained from one 300-foot well drilled in 1922; due to perforated casing water is obtained from both strata. Same water supply is used in adjoining Texico, N. Mex.	"A" sample represents all children in fourth, fifth, sixth, and seventh grades who used city water continuously.
Lockney (1,466)	30	0	1	2	6	2	0	0	0	0	8	9	2	0	0	Obtained from one 120-foot well drilled in 1928, cased to the first stratum. Prior to 1928 municipal supply from a well same depth but not cased.	"B" sample represents children using windmill well water continuously from birth in Lockney and immediate rural district.

[1] None examined.

[2] Rand McNally pocket map of Texas, 1934.

TABLE 1.—*Summary of mottled enamel findings and history of water supply in certain cities of (1) Panhandle and west Texas and (2) east central Texas*—Continued

1. Panhandle and west Texas—Continued

City and population (census of 1930)	Total number of children examined	Children classified according to mottled enamel diagnosis														History of water supply	Remarks
		(A) Continuous residence with constant use of city water							(B) Changes in residence and/or water history								
		Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe	Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe		
Tahoka (1,620)	58	7	6	14	11	7	0	0	2	2	0	3	5	1	0	From 11 wells 80 to 100 feet deep obtaining water from first stratum. First well drilled 1924; subsequent wells added as needed; 9 wells are "gravel backed."	"A" sample, all children in second, third, fourth, fifth, and sixth grades using city water continuously since birth. "B" sample, children from immediate rural district.
Stanton (1,384)	14	0	3	2	7	2	0	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	From 3 wells, 2 drilled 1927, 1 in 1930, each 135 feet; first stratum cased off. No common water supply prior to 1927.	Sample consists of children in fourth, fifth, and sixth grades using municipal water continuously since 1927.
Perryton (2,824)	137	3	0	8	18	3	0	0	38	9	33	21	4	0	0	From two 400-foot "gravel backed" wells installed 1927. City water is a composite from all 3 strata. Municipal supply in use since 1923.	Entire fifth and sixth grades, Perryton and rural school district.
Odessa (2,407)	32	7	1	7	3	1	0	0	7	1	1	3	1	0	0	From six 140-foot wells: 5 drilled 1928, 1 in 1933. No city water prior to 1928.	Both samples from third and fourth grades. "A" used city water constantly past 6 years. "B" used individual wells constantly since birth.
Spearman (1,580)	107	2	1	5	8	1	0	0	48	8	27	9	1	0	0	From two 380-foot wells drilled 1924 and 1927. Stratum from which water is obtained is unknown.	All of fifth and sixth grades examined, Spearman and rural school district.
Pecos (3,304)	51	20	11	16	3	1	0	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	From 2 wells 280 feet deep drilled 1914. The first stratum is cased off.	Sample consists of all children in third, fifth, sixth, and seventh grades using city water exclusively since birth.
Canyon (2,821)	80	2	3	9	5	0	0	0	15	6	15	16	16	2	0	Since 1928 from 500-foot well of West Texas Utility Co. Prior to 1928 from 4 city wells, 250 to 500 feet. No data procured on stratum or strata from which these waters were and are obtained.	In sample "B" there were 14 children from immediate rural district who always used water from individual windmill wells. They show a more severe type of mottled enamel than those using Canyon city water.
Dimmitt (829)	53	5	1	3	3	0	0	0	12	6	6	10	5	2	0	From one 230-foot well drilled 1927. No data on stratum from which water is obtained. Prior to 1927 there was no common water supply.	"A" sample represents all children in fifth and sixth grades using city water constantly for past 6 years. In sample "B" there were 9 children from immediate rural district who from birth continuously used water from individual wells. All showed a more severe type of mottled enamel than those using Dimmitt city water.
Dalhart (4,691)	248	20	8	27	15	0	0	0	100	8	43	22	5	0	0	From 2 wells 340 feet deep, drilled 1928. Prior 1928 water obtained from wells of similar depth.	Examinations were made of children in fourth, fifth, and sixth grades.
Wink (3,963)	16	4	2	7	3	0	0	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	From 5 wells 220 feet deep, drilled 1927; first stratum is cased off. At present only 1 well is being used. No common water supply prior 1927.	Sample taken from fourth grade, none over 10 years of age and had used city water for at least past 6 years.
Stratford (873)	76	7	1	10	4	0	0	0	27	10	10	6	1	0	0	From the 302-foot well, drilled 1930, and owned by the West Texas Utility Co. No information obtained concerning previous municipal supply.	Examinations included all children of fourth, fifth, and sixth grades.
Fort Stockton (2,695)	13	4	3	4	2	0	0	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	From 175-foot well, drilled 1927. Between 1923–30 water was obtained from 2 wells 306 and 365 feet. These wells were abandoned in 1930. Between 1927–30 the supply was composite of all wells.	Sample represents all children in fourth fifth, and sixth grades who used city water continuously.
Dumas (700) [2]	86	7	3	16	4	0	0	0	39	4	9	3	1	0	0	From 2 wells 530 and 550 feet, installed in 1930. No information regarding stratum from which water is obtained.	All children in the fourth and fifth grades were examined.
Panhandle (2,035)	42	7	0	8	1	0	0	0	10	5	8	5	0	0	0	From 2 wells each 550 feet installed 1927. No information on stratum from which water is obtained. No common water supply prior 1927.	All children in sixth grade examined. "A" sample represents children using city water for the past 6 years.
Pampa (10,470)	117	27	12	9	1	0	0	0	46	9	10	3	0	0	0	Present supply from 3 wells, 384 feet, cased entire depth, and taking water from third stratum. There are 6 other wells not being used at present.	All children in sixth grade examined. "A" sample represents children using city water for the past 6 years or longer.
Borger (6,532)	124	29	6	8	1	0	0	0	57	10	10	3	0	0	0	From wells 250 to 500 feet, located 18 miles south in Carson County (Plain Station) and installed 1928. No common water supply prior 1928.	All of fifth and sixth grades examined. "A" sample represents children using city water for the past 6 years.
Big Spring (13,735)	63	58	3	2	0	0	0	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	Municipal supply consists of 23 wells. One-third of supply from 13 wells 200 feet, drilled in 1923. Two-thirds of supply from 8 wells 280 to 300 feet, drilled 1927. Between 1894–1923 all municipal water from "Old Park" supply, which was supplemented until 1927 with 1923 group of wells. Between 1927–33 "Old Park" supply shut down. Between November 1933 and May 1934, 1927 group of wells also shut down. Wells located 2, 6, and 9 miles, respectively, south of Big Spring.	Sample consists of all children in fifth and sixth grades whose history indicated constant residence and continuous use of city water.
Total	2,803	236	85	264	325	322	80	6	637	123	367	283	152	23	0		

(1) None examined.

[2] Rand McNally pocket map of Texas, 1934.

TABLE 1.—*Summary of mottled enamel findings and history of water supply in certain cities of (1) Panhandle and west Texas and (2) east central Texas*—Continued

2. East central Texas

City and population (census of 1930)	Total number of children examined	Children classified according to mottled enamel diagnosis														History of water supply	Remarks
		(A) Continuous residence with constant use of city water							(B) Changes in residence and/or water history								
		Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe	Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe		
Bartlett (1,873)	29	0	0	4	3	18	3	1	(1)	(1)	(1)	(1)	(1)	(1)	(1)	From a 2,005-foot well drilled 1901 and in constant use since. Apparently obtaining water from different strata.	Sample consists of all children in fifth, sixth, and seventh grades with a continuous residence since birth and constant use of city water.
Italy (1,230)	34	0	2	6	11	10	5	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	From 850-foot well drilled 1912 and in constant use since. According to local data water is obtained from second Woodbine stratum.	Sample consists of all children in fourth, fifth, sixth, and seventh grades who comply with "A" classification.
Frost (748)	13	0	1	4	3	5	0	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	From 1,184-foot well drilled 1903 into the Trinity sands. This is the only supply used since that year.	Sample consists of all children in fifth, sixth, and seventh grades who comply with "A" classification.
Taylor (7,463)	45	5	3	14	10	8	5	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	From a 3,260-foot well drilled 1913 and a 3,300-foot well drilled 1934. Due to high sulphur content of city water some inhabitants use cistern water for certain domestic purposes.	Sample consists of all children in the sixth grade coming under "A."
Palmer (758)	16	1	2	3	6	2	2	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	Between 1900 and 1928 supply obtained from 1,170-foot well; 1928 to date, entire city supply obtained from 1,172-foot well drilled into Woodbine stratum.	Sample consists of all children in the third, fourth, and fifth grades who comply with "A" classification.
Ferris (1,438)	26	3	2	5	10	5	1	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	Since 1921 entire city water supply obtained from a 1,400-foot artesian well.	Sample consists of all children in the fourth, fifth, and sixth grades who comply with "A" classification. During the examination in Ferris, 5 pupils who had always lived in the nearby community of India, but who attended school in Ferris, were observed. All 5 showed mottled enamel moderate in severity. These 5 are not included in the Ferris totals.
Belton (3,779)	43	6	5	6	17	7	2	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	From 2 wells, 1 drilled 1903, 980 feet in the first Trinity sands, and 1 drilled 1915, 1,180 feet into the second Trinity sands.	Sample consists of all children in the fifth and sixth grades who comply with "A" classification.
Ennis (7,069)	71	8	6	21	23	10	3	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	From 1909–25 supply obtained from two 1,325-foot wells drilled to the first Woodbine sand. August 1925 well installed, obtaining water from the second Woodbine sand between 1,700 and 1,798 feet deep; 1930 the smaller of the first 2 wells was abandoned. Present supply comes from one 1,325-foot well and one 1,798 feet, the latter in the second Woodbine stratum, and furnishing the major portion of the water supply since 1926.	Sample consists of all children in the third, fourth, and fifth grades who comply with "A" classification.
Gatesville (2,601)	17	2	2	6	6	1	0	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	From two 700-foot wells drilled in 1910 and in constant use since that date.	Sample consists of all children in the sixth grade who comply with "A" classification.
Granger (1,703)	12	0	1	5	6	0	0	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	From a 2,531-foot well drilled 1906 or 1907 and in constant use since that year.	Sample consists of all children in the sixth and seventh grades who comply with "A" classification.
Waxahachie (8,042)	30	11	5	11	3	0	0	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	Since 1913 from 3 wells, 2,900 to 3,000 feet, drilled into the Trinity sands. These wells were drilled 1913, 1925, and 1931, respectively.	Sample represents children from the fourth, sixth, and seventh grades of the Central Ward School who comply with "A" classification.
West (1,807)	25	11	6	7	1	0	0	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	From a well, 2,200 feet deep, drilled 1894 into the second Trinity sands and in continuous use since that year.	Sample consists of all children in the fifth and sixth grades who comply with "A" classification.
Hillsboro (7,823)	46	19	13	12	2	0	0	0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	Since 1923 city water supply obtained from 14 wells. There is considerable variation in the depth and strata from which the water is and was obtained. Limited space precludes detailed description of this supply.	Sample consists of all children in the sixth grade who comply with "A" classification.
Total	407	66	48	104	101	66	21	1	(1)	(1)	(1)	(1)	(1)	(1)	(1)		
Grand total (1 plus 2).	3,300	292	133	368	426	388	101	7	637	123	367	283	152	23	0		

[1] None examined.

[2] Rand McNally pocket map of Texas, 1934.

TABLE 2.—*Summary of mottled enamel findings in certain communities of the Panhandle and west Texas with a common water supply but where an insufficient number of examinations precluded the computation of a mottled enamel index*

City and population (census of 1930)	Total number of children examined	Children classified according to mottled enamel diagnosis														History of water supply	Remarks
		(A) Continuous residence with constant use of city water							(B) Changes in residence and/or water history								
		Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe	Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe		
Lorenzo (739)	25	0	0	1	4	2	0	0	2	4	4	4	3	1	0	Municipal water supply obtained from one 280-foot well, drilled in 1926. First stratum cased off.	Sample "A" represents all children in the third and fourth grades who used city water for the past 6 years. Sample "B" represents children who have used continuously water from local or nearby rural windmill wells.
Idalou (538)	33	0	0	1	2	3	2	0	1	1	5	6	12	0	0	From 1 well, drilled in 1925, 136 feet. First stratum cased off and city supply obtained from second stratum.	Sample "A" represents children from the second, third, fourth, and fifth grades. Sample "B" represents children who have used continuously water from nearby rural windmill wells.
White Deer (1,010)	28	6	0	0	0	0	0	0	18	0	3	1	0	0	0	From one 396-foot well, drilled in 1926, cased entire depth and obtaining water from the "bottom" stratum only.	Sample "A" represents all children of the sixth grade coming under that classification. High percentage of children showing normal calcification warrants a detailed epidemiological survey.
Vega (519)	37	0	0	1	3	2	0	0	10	2	8	9	2	0	0	From 2 wells, one 240-foot, drilled in 1921, and one 396-foot, drilled in 1928. The latter supplies practically all the water.	Sixth and seventh grades examined. "B" includes children from immediate rural districts.
Total	123	6	0	3	9	7	2	0	31	7	20	20	17	1	0		

TABLE 3.—*Summary of mottled-enamel findings in certain communities of the Panhandle and west Texas with no common water supply. (See two exceptions under "Remarks")*

Communities where water from individual windmill wells is used

City and population (census of 1930)	Total number of children examined	Children classified according to mottled enamel diagnosis							History of water supply and remarks
		Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe	
Southland (400)[1]	21	0	0	0	4	12	5	0	Sample represents all children in fourth and fifth grades who stated they had either lived in Southland or immediate rural school district all their lives and had always used water from windmill wells. Individual windmill wells in this particular area are approximately 100 to 125 feet in depth.
Two Floyd County rural districts.	23	0	2	6	7	7	1	0	Examinations were made in 2 rural schools, one 4 miles south of Lockney, the other 12 miles west and 2 miles south of Floydada; 14 of the children stated they had always used water from individual windmill wells located within the borders of their respective school districts.
Three Potter County rural districts.	98	22	8	16	35	16	1	0	Examinations were made in 3 rural schools, located at Bushland, River Road, and Highland Park, respectively, 10 miles west, 6 miles north, and 10 miles east of Amarillo. Sample contains children with a history of continuous residence in the district and others born elsewhere. Water is obtained from individual windmill wells apparently from the first stratum.
Happy (724)	29	5	6	7	5	5	1	0	Although Happy has a municipal water supply installed in 1928, it was not possible to find more than 3 children in the grades examined who had used the city water for at least the past 5 years, many of the inhabitants continuing to use windmill wells. Most of the sample represents children from the immediate rural districts.
Monroe (50)[1]	17	3	6	6	2	0	0	0	Children in this sample stated they had lived continuously either in Monroe or the immediate rural district and had used water from individual windmill wells.
Abernathy (858)	34	9	12	7	4	1	1	0	There is no municipal water supply in Abernathy and sample represents children who stated they had always lived either in Abernathy or in the school district, and who had always used water from individual windmill wells, which vary from 118 to 130 feet in depth.
Wildorado (105)[1]	30	6	10	3	10	1	0	0	This sample represents children from the third, fourth, fifth, sixth, and seventh grades and contains many with both continuous and variable histories. Individual windmill wells are approximately 150 feet deep.
Farnsworth (25)[1]	20	7	2	4	4	3	0	0	Sample represents children in the fourth, fifth, sixth, and seventh grades and includes children from both Farnsworth and the immediate rural district. Water histories in this group vary. Water is obtained from individual windmill wells.

[1] Rand McNally pocket map of Texas, 1934.

TABLE 3.—*Summary of mottled-enamel findings in certain communities of the Panhandle and west Texas with no common water supply. (See two exceptions under "Remarks")*—Continued

Communities where water from cisterns is used for domestic purposes

City and population (census of 1930)	Total number of children examined	Children classified according to mottled enamel diagnosis							History of water supply and remarks
		Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe	
Jayton (623)	6	6	0	0	0	0	0	0	The 6 examined represent children who had lived in Jayton all their lives and had used continuously water from cisterns for domestic purposes. Afternoon school dismissal of the pupils prevented examination of any additional number. The city has a common water supply, but it is of a type which obviates its use for domestic purposes, and the inhabitants of this town use cistern water for cooking and drinking.
Gail (100)[1]	17	15	1	1	0	0	0	0	Sample represents children from the fourth, fifth, and sixth grades with a history of continuous residence in Gail or nearby rural districts for the major part of their lives. The 1 case of mottled enamel had lived at O'Donnell, an endemic area, from birth to 2 years of age. Water for domestic purposes is obtained from cisterns and occasionally surface sources.
Total	295	73	47	50	71	45	9	0	

[1] Rand McNally pocket map of Texas, 1934.

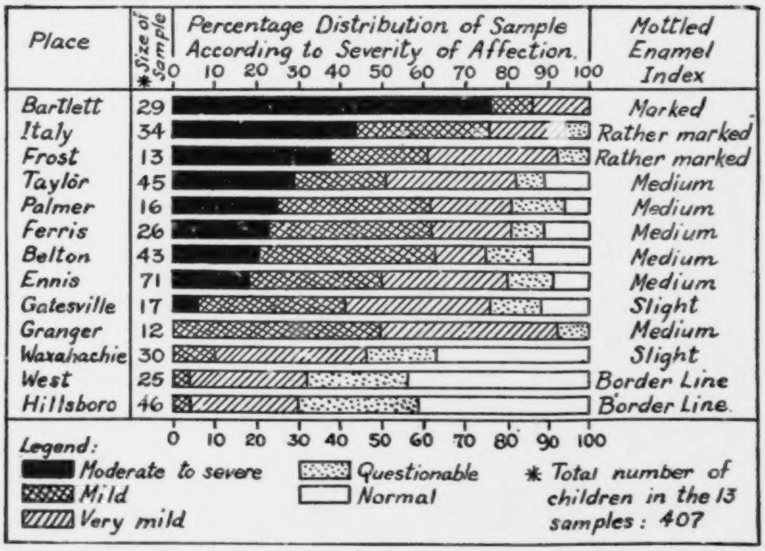


FIGURE 12.—*Severity of mottled enamel in children of certain selected places of east central Texas*

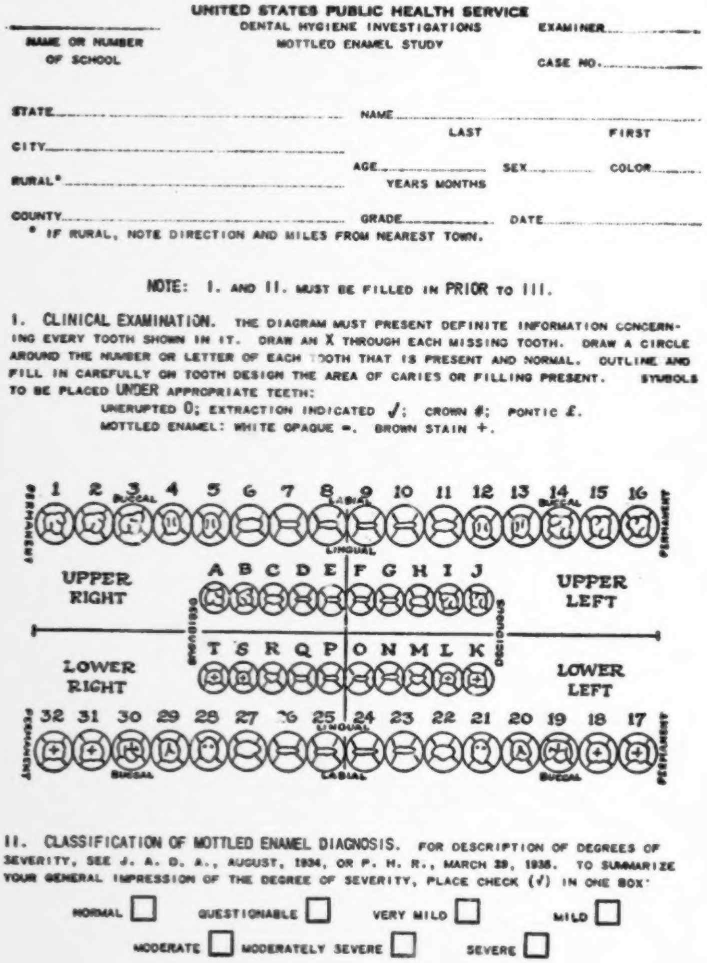
(Children in continuous residence and uninterrupted use of municipal water)

STUDIES ON THE MINIMAL THRESHOLD OF THE DENTAL SIGN OF CHRONIC ENDEMIC FLUOROSIS (MOTTLED ENAMEL)

By H. TRENDLEY DEAN and ELIAS ELVOVE

Since the publication of the results of three independent studies in 1931 (*1*), (*2*), (*3*), associating the presence of fluoride in the drinking water with the endemic hypoplasia of the permanent teeth known as mottled enamel, the question of what constitutes a permissible amount of fluoride in a domestic water supply frequently arises. Attempts to prevent the further development of this disease by removing the toxic amounts of fluorides present in the water is naturally dependent upon reliable and definite information concerning this permissible maximum, or minimal threshold.

For public health purposes we have arbitrarily defined the minimal threshold of fluoride concentration in a domestic water supply as the highest concentration of fluoride incapable of producing a definite degree of mottled enamel in as much as 10 percent of the group examined. This group should consist of at least 25 children 9 years of age or older, who, since birth, had continuously used the water under investigation for both drinking and cooking. A community is given a "negative" mottled-enamel index when "less than 10 percent of the children show 'very mild' or more severe types of mottled enamel" (Ref. 8.) In cases where the examination of the first 25 children discloses an incidence of less than 75 percent it has been found desirable, if not necessary, to increase the number in the group to 50 or more. In other words, when more than 25 percent are diagnosed as "normal" or "questionable," the increase in the number of the group examined is necessary to compensate for fluctuations in sampling and their possible effect on the computation of the community mottled-enamel index. The thorough surveys thus far made, though limited in number, have indicated that, when an adequate number of children are examined, there is orderly uniformity in the group response to the fluoride concentration, with regard both to the incidence and the percentage distribution of severity, particularly the latter.



UNITED STATES PUBLIC HEALTH SERVICE
DENTAL HYGIENE INVESTIGATIONS
MOTTLED ENAMEL STUDY

NAME OR NUMBER OF SCHOOL ____________

EXAMINER ____________

CASE NO. ____________

STATE ____________

NAME ____________ LAST ____________ FIRST

CITY ____________

AGE ____________ YEARS MONTHS

SEX ____________

COLOR ____________

RURAL* ____________

COUNTY ____________

GRADE ____________

DATE ____________

* IF RURAL, NOTE DIRECTION AND MILES FROM NEAREST TOWN.

NOTE: I. AND II. MUST BE FILLED IN PRIOR TO III.

I. CLINICAL EXAMINATION. THE DIAGRAM MUST PRESENT DEFINITE INFORMATION CONCERNING EVERY TOOTH SHOWN IN IT. DRAW AN X THROUGH EACH MISSING TOOTH. DRAW A CIRCLE AROUND THE NUMBER OR LETTER OF EACH TOOTH THAT IS PRESENT AND NORMAL. OUTLINE AND FILL IN CAREFULLY ON TOOTH DESIGN THE AREA OF CARIES OR FILLING PRESENT. SYMBOLS TO BE PLACED UNDER APPROPRIATE TEETH:

UNERUPTED 0; EXTRACTION INDICATED √; CROWN #; PONTIC £.
MOTTLED ENAMEL: WHITE OPAQUE =. BROWN STAIN +.

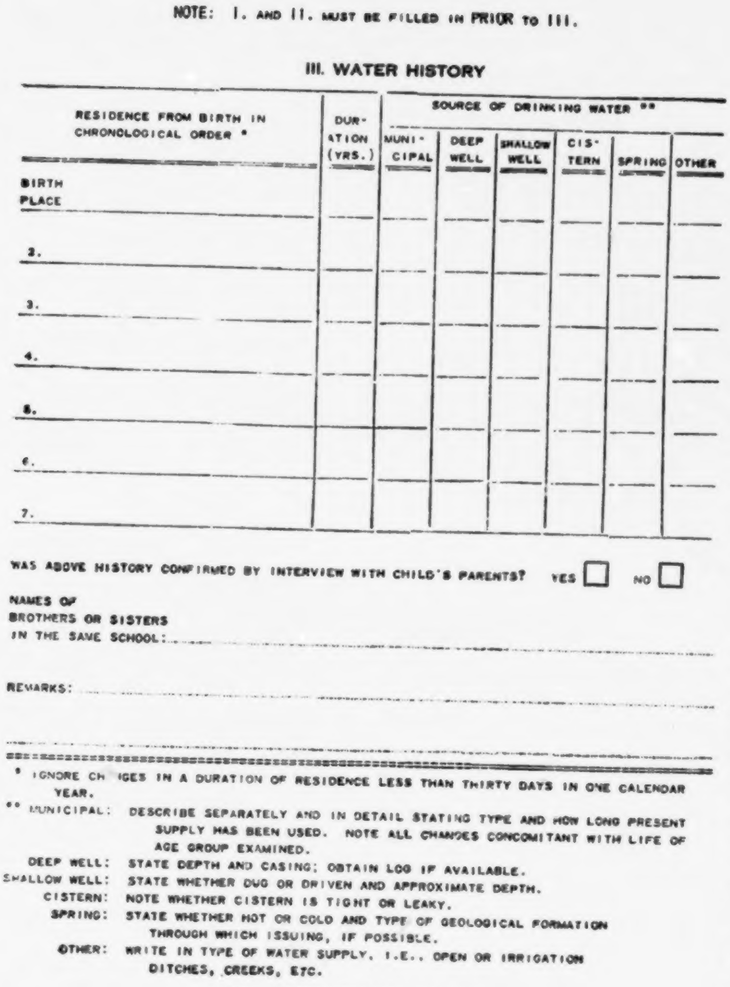
II. CLASSIFICATION OF MOTTLED ENAMEL DIAGNOSIS. FOR DESCRIPTION OF DEGREES OF SEVERITY, SEE J. A. D. A., AUGUST, 1934, OR P. H. R., MARCH 29, 1935. TO SUMMARIZE YOUR GENERAL IMPRESSION OF THE DEGREE OF SEVERITY, PLACE CHECK (√) IN ONE BOX:

NORMAL ☐ QUESTIONABLE ☐ VERY MILD ☐ MILD ☐
MODERATE ☐ MODERATELY SEVERE ☐ SEVERE ☐

FIGURE 1 (a).—Face of record form.

An attempt to determine the minimal threshold by a correlation of clinical observations with chemical findings is obviously unwarranted until certain variables intimately associated with the problem are removed. Chief of these variables are (*a*) discontinuities in time of exposure, and (*b*) changes in the fluoride content of water supplies.

In considering the time variable it must be borne in mind that the causative factor of mottled enamel is probably operative during the entire period of calcification of the permanent teeth. An observed effect, therefore, may be the result of a comparatively low fluoride concentration operating during the entire period, or a somewhat higher concentration for a shorter period. In other words, in endemic areas where the common water supply contains fluoride in concentrations only slightly above the minimal threshold, the time of risk of exposure would probably have to be continuous with the period of calcification. On the other hand, in areas where water of a high fluoride concentration is used, its use during a fractional portion of the period of calcification is sufficient to produce the signs of mottled enamel. A careful analysis of the Bauxite survey (*4*) illustrates this point. Hence, in order to eliminate the influence of this variable, clinical observations were made only on those children whose histories showed that the water in question was used continuously since birth for both drinking and cooking. Breaks in continuity



NOTE: I. AND II. MUST BE FILLED IN PRIOR TO III.

III. WATER HISTORY

RESIDENCE FROM BIRTH IN CHRONOLOGICAL ORDER *	DURATION (YRS.)	SOURCE OF DRINKING WATER **					
		MUNICIPAL	DEEP WELL	SHALLOW WELL	CISTERN	SPRING	OTHER
BIRTH PLACE							
2.							
3.							
4.							
5.							
6.							
7.							

WAS ABOVE HISTORY CONFIRMED BY INTERVIEW WITH CHILD'S PARENTS? YES ☐ NO ☐

NAMES OF BROTHERS OR SISTERS IN THE SAME SCHOOL: ....

REMARKS: ....

* IGNORE CHANGES IN A DURATION OF RESIDENCE LESS THAN THIRTY DAYS IN ONE CALENDAR YEAR.

** MUNICIPAL: DESCRIBE SEPARATELY AND IN DETAIL STATING TYPE AND HOW LONG PRESENT SUPPLY HAS BEEN USED. NOTE ALL CHANGES CONCOMITANT WITH LIFE OF AGE GROUP EXAMINED.

DEEP WELL: STATE DEPTH AND CASING; OBTAIN LOG IF AVAILABLE.

SHALLOW WELL: STATE WHETHER DUG OR DRIVEN AND APPROXIMATE DEPTH.

CISTERN: NOTE WHETHER CISTERN IS TIGHT OR LEAKY.

SPRING: STATE WHETHER HOT OR COLD AND TYPE OF GEOLOGICAL FORMATION THROUGH WHICH ISSUING, IF POSSIBLE.

OTHER: WRITE IN TYPE OF WATER SUPPLY, I.E., OPEN OR IRRIGATION DITCHES, CREEKS, ETC.

FIGURE 1 (b).—Back of record form.

not exceeding a total of 30 days in any one calendar year are excepted.

The other major variable relates to changes in the fluoride content of the water supply. Any attempt to correlate clinical observations with a single fluoride determination of a common water supply associated with chronic endemic dental fluorosis clearly introduces a possible error. While it is true that fluctuations in mineral content of a water are least when the water is obtained from deep wells, nevertheless there may be changes in the physical set-up or source of the municipal water supply during the child's lifetime which would cast doubt upon any correlations which might be made. Stating it differently, the amount of fluoride in a water sample taken at the time of a clinical survey of a group, for instance, of 12-year-old children, would be of little value unless the water supply had undergone no changes during a period concomitant with the life of the group of children examined.

Furthermore, when dealing with surface waters or shallow wells the seasonal and annual rainfall and other meteorological conditions introduce factors which would make it impossible to draw reliable conclusions from a single fluoride determination of the water in question. In this report the mean annual fluoride content, later referred to, represents an arithmetical mean of 12 consecutive monthly samples.

Eleven cities were chosen for study. In six of these mottled enamel was known to be endemic (*5*); five others, with as nearly as possible comparable conditions, but reported as nonendemic, were selected for "controls." These cities contained populations sufficiently large to permit the necessary sifting and elimination of those children whose histories failed to show an exclusive use of the municipal water from birth. Six of the cities have populations (census of 1930) in excess of 10,000, the largest being more than 50,000. From each of these cities 12 consecutive monthly water samples of the municipal water supply were collected and forwarded to the National Institute of Health for fluoride determinations.

A report of a survey of four of these cities, prefaced by a description of their municipal water supply follows. These four cities are Colorado Springs, Colo.; Monmouth and Galesburg, Ill.; and Pueblo, Colo.

DESCRIPTION OF THE MUNICIPAL WATER SUPPLIES

Colorado Springs, Colo.—The municipal water supply of Colorado Springs is obtained from surface sources; namely, melted snow from the south, west, and east slopes of Pike's Peak, and the east and west slopes of Mount Baldy. The water is stored in a system of seven mountain reservoirs, located at altitudes ranging from 9,300 to 12,000 feet. From this chain of reservoirs the water is conveyed through a transmission system to the settlers at Manitou, thence by gravity to three distributing reservoirs known as the "High Line," "Mesa No. 1," and "Mesa No. 2." These distributing reservoirs are located on a mesa just west of the city, and from these reservoirs begin the city distribution system and service mains. Water impounded in both the High Line and the Mesa Reservoirs is obtained from a common source and represents the type of water used by the inhabitants of the city for at least the past 20 years. The only exception to the foregoing is that in the extreme southwestern panhandle-like projection of the westward city limits, a few lateral distribution mains lead off from the Bear Creek pipe line before it connects with the transmission lines between Manitou and the Mesa Reservoirs. A small fraction of the population is served in this manner; the source, however, of the Bear Creek water is not far (½ mile) from Lake Moraine, one of the main reservoirs in the city mountain system. Because of this possible variable, children residing in this section of the city were not included in the group upon which the mottled enamel index was based.

Monmouth, Ill.—The municipal water supply of Monmouth is obtained from two wells 2,445 feet in depth. The first well was completed early in 1925, the second in 1926. Both wells obtain their

TABLE 1.—*Summary of data with relation to continuity of residence and concomitant use of the municipal water*

	Colorado Springs	Monmouth	Galesburg	Pueblo (north of Arkansas River)
1. Number of grade public schools in city	10	4	9	8
2. Number of grade public schools in which examinations were held	5	4	4	4
3. Number of 9-year old pupils in attendance in (2) on date of examination	253	142	187	215
4. Number of pupils in (3) whose histories on questioning indicated constant residence and concomitant use of municipal water and who were examined	79	38	57	83
5. Percentage of age group present examined under (4)	31.2	26.7	30.5	[1] 38.6
6. Number of schedules eliminated by house-to-house recheck	25	9	18	34
7. Percentage of total present showing constant residence and water history	21.3	20.4	20.8	22.8

[1] This percentage is slightly higher than the others because of the dual water supply in Pueblo. Certain children were doubtful whether they had used the North Pueblo supply exclusively, but were nevertheless examined. When the house-to-house recheck revealed use of the South Pueblo water supply during some period of their life, these schedules were eliminated.

TABLE 2.—*Distribution according to severity*[1] *of those examined in (4), table 1*

City	Number of children	Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe	Incidence
		Number							
Colorado Springs	79	15	11	24	14	13	2	0	67.1
Monmouth	38	14	8	14	2	0	0	0	42.1
Galesburg	57	26	11	15	3	2	0	0	35.1
Pueblo	83	73	8	2	0	0	0	0	2.4
		Percent							
Colorado Springs	79	19.0	13.9	30.4	17.7	16.5	2.5	0	67.1
Monmouth	38	36.8	21.0	36.8	5.3	0	0	0	42.1
Galesburg	57	45.6	19.3	26.3	5.3	3.5	0	0	35.1
Pueblo	83	88.0	9.6	2.4	0	0	0	0	2.4

TABLE 3.—*Distribution according to severity*[1] *of those remaining after recheck (6), table 1*

City	Number of children	Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe	Incidence
		Number							
Colorado Springs	54	10	8	14	10	11	1	0	66.6
Monmouth	29	10	5	12	2	0	0	0	48.3
Galesburg	39	16	8	11	3	1	0	0	38.5
Pueblo	49	44	3	2	0	0	0	0	4.0
		Percent							
Colorado Springs	54	18.5	14.8	25.9	18.5	20.4	1.8	0	66.6
Monmouth	29	34.5	17.2	41.4	6.9	0	0	0	48.3
Galesburg	39	41.1	20.5	28.2	7.7	2.6	0	0	38.5
Pueblo	49	89.8	6.2	4.0	0	0	0	0	4.0

[1] For a detailed description of the various gradations of mottled enamel severity, see reference 8.

water from the "Potsdam" stratum of the Cambrian sandstone. During the first 6 or 8 months of 1925 some water from the old wells in the St. Peter sandstone was added to that obtained from the first well, temporarily constituting a mixed supply. The percentage of the municipal water obtained from the old wells is not known, as the pumping records were not available. The second Cambrian well was completed early in 1926, and since that date all municipal water has been obtained from these two wells.

Both wells have 90 feet of 20-inch copperoid casing, 400 feet of 18-inch cast-iron casing, and 1,200 feet of 12-inch cast-iron casing. Water from the St. Peter sandstone, which is found between 1,100 and 1,250 feet in this locality, is apparently cased off. No strainers which permit mixture of water from higher strata are present in the casing. There has been practically no change in the water level of either well since installation. Water from these two wells is more than ample for municipal needs; one well is pumped during the day and the other during the night.

Galesburg, Ill.—The public water supply of Galesburg is obtained from two 2,414-foot wells drilled to the Cambrian sandstone. The first well was drilled in 1919, installed in 1920, and has been in continuous use since. In 1928 a second well of the same depth was completed. The casing record of both wells indicates that water from the St. Peter sandstone is completely cased off. Since 1928 these two wells have furnished practically all (more than 98 percent) of the water used by the population.

Between 1924 and 1928 the common water supply consisted of approximately 60 percent from the first "Potsdam" (2,414-foot) well and 40 percent from wells in the St. Peter sandstone. The latter wells (Central Fire Station and Brooks Street Station) were drilled in 1917 and 1918, and are 1,252 and 1,245 feet deep, respectively. Both of these latter wells are cased to the St. Peter sandstone so as to exclude water from higher levels. Water history prior to 1924 is omitted, as the time would be prior to the year of birth of the group of children examined and, therefore, not relevant.

Pueblo, Colo.—The municipal water supply of Pueblo is obtained from surface sources, namely, the Arkansas River. The city of Pueblo has two water systems, all of the city north of the Arkansas River being supplied by what is known as the "Pueblo Water Works, District No. 1," or "North Pueblo water supply," while that half of the city located south of the Arkansas River obtains its water from another system known as the "Pueblo Water Works, District No. 2," or "South Pueblo water supply." Both systems, however, obtain water from the Arkansas River.

TABLE 4.—*Fluoride (F) content of monthly samples*

Month	Colorado Springs	Monmouth	Galesburg	Pueblo
	Parts per million			
1933				
November	2.8	1.6	1.8	0.6
December	2.9	1.6	1.8	.6
1934				
January	2.9	1.6	1.8	.6
February	3.0	1.7	1.9	.6
March	3.0	1.7	1.9	.6
April	3.0	1.7	1.9	.7
May	2.9	1.9	1.8	.6
June	2.3	1.7	1.9	.3
July	2.0	1.8	2.0	.5
August	1.8	1.8	1.9	.6
September	1.9	1.8	1.9	.6
October	2.0	1.6	1.8	.6
Mean annual fluoride content	2.5	1.7	1.86	0.57

The monthly water samples collected by the Pueblo City Health Department were all of the North Pueblo supply. Consequently the clinical examinations were limited to the children residing in that half of the city. A description of the North Pueblo supply follows:

Water is taken from the Arkansas River about 3 miles west of the city and diverted into reservoirs. In 1925 there were six reservoirs, and in 1928, one more was added. Treatment consists of preliminary sedimentation, coagulation with aluminum sulphate, followed by sedimentation and disinfection with ammonia-chlorine. Reservoirs nos. 1, 2, 3, and 4 are used for preliminary sedimentation, and reservoirs nos. 5, 6, and 7 for sedimentation after coagulation. The treatment does not include filtration. Prior to 1928 iron sulphate and lime were used as a coagulant, and prior to November 1931 chlorine was used without ammonia.

The North Pueblo water department has been obtaining water from this same source for the last 46 years. It might also be noted that between November 1933 and October 1934, the period during which samples were taken, there was an exceptionally low flow of the Arkansas River.

METHOD OF CLINICAL SURVEY

In order to study clinical conditions in a group whose water history showed exclusive use of the water supply upon which the chemical determinations were made, examinations were limited to the 9-year-age group present in the school on the date of the examination. The selection of the 12-year group would have been more desirable, as practically all permanent teeth excepting the third molars are present in the mouth at that age. However, the present Monmouth water supply had been in use only 9 years at the time of the examination, and it was deemed advisable to limit the examination to the age group that had used the present supply exclusively since birth rather than introduce an additional variable by selecting a higher age group. The same age group was then selected at Colorado Springs and Pueblo in order that the resultant data might be comparable. Brief reference will be made later to a variable present in the Galesburg water supply between 1924 and 1928.

The examinations were conducted in the following manner: First, all children who stated that they had not lived in the city continuously (30 days in a calendar year excepted) or had not used the municipal water supply exclusively were eliminated. The remainder were then called into the office of the school principal, one or two at a time, and further questioned as to their residence and water history. This cross-questioning often revealed breaks in continuous residence such as living a year or more in some nearby town or vacation trips in excess of 30 days. In addition there were a few instances of children who had lived in the community continuously but who had obtained their domestic water from other than the public supply. Children coming under these classifications were eliminated from further study.

If the questioning of the child elicited a history of continuous residence in the city and an exclusive use of the municipal water, the examination continued. The findings were recorded on a special card designed for mottled enamel surveys (fig. 1). The examinations were made in a good light with the child seated facing a window. Mouth mirrors, free of blemishes, and new explorers SSW no. 5 or equal, were utilized in making the examination. In addition to mottled enamel, other defects of the enamel such as caries, present or past (fillings), pits and fissures, hypoplasias, etc., were recorded.

Following the examination, the home of each child was visited, and the information recorded in fig. 1 under "III. Water History" was carefully rechecked by an interview with the child's parent. This recheck revealed additional inaccuracies in residence or water supplies which the child either did not know or had forgotten. Under the conditions of this survey it was possible to find only approximately 20 percent in this age group who could demonstrate a continuous residence and an exclusive use of the city water during their lifetime. The results obtained are summarized in tables 1, 2, and 3.

In evaluating the Galesburg study, the variable in the city water supply between 1924 and 1928 must be borne in mind. However, it should be noted, parenthically that of the 15 individuals showing mottled enamel among the 39 rechecked cases, 11 of them had no erupted bicuspids. In the remaining four with bicuspids present, mottled enamel was observed in the bicuspids of each individual. A resurvey of Galesburg should be made 4 to 5 years later in order to determine the actual mottled enamel index.

As has been noted previously, samples of the waters were obtained monthly. The fluoride content was estimated colorimetrically by means of the zirconium-alizarin reagent (*6*). The results obtained are given in table 4.

While it may seem reasonable that the mottled enamel index will be found to depend entirely on the fluoride concentration of the drinking water, it is also possible that other constituents of the water may have some influence on the activity of the fluoride. For this reason we believe that a careful survey of a community for mottled enamel should, for the present at least, include also a chemical analysis of the drinking water for constituents other than fluoride.

Results of the chemical analyses of the waters[*] are given in table 5.

DISCUSSION

An analysis of the field data indicates the care that must be exercised in checking the continuity of residence and water history of the children under examination. As a result of checking the water histories as given by the child by a followup recheck with the parents, only about 20 percent of the children in the age group studied and present in the

Table 5.—*Analyses of the waters used*

	Colorado Springs	Monmouth	Galesburg	Pueblo (north of Arkansas River)
	Parts per million			
Residue on evaporation	46.5	1,031.5	1,080.0	588.0
Loss on ignition	9.0	62.7	51.2	75.0
Fixed residue	37.5	968.8	1,028.8	513.0
Silica (SiO_2)	16.0	14.0	12.8	20.0
Iron (Fe)	.12	.18	.15	.14
Aluminum (Al)	.5	.3	.3	0
Calcium (Ca)	7.2	69.3	62.2	83.6
Magnesium (Mg)	.6	26.6	25.7	27.3
Sodium and potassium (calculated as Na)	5.4	249.9	288.8	54.9
Bicarbonate (HCO_3)	24.4	283.0	296.5	187.8
Sulfate (SO_4)	4.5	412.1	341.4	244.5
Nitrate (NO_3)	1.0	3.3	3.3	5.2
Chloride (Cl)	1.0	117.0	196.0	16.5
Fluoride (F)	2.0	1.8	1.9	.6
Phosphate (PO_4)	0	0	0	0
Boron (B)	0	.8	.9	0

[a] These samples of water from Monmouth and Galesburg, Ill., were received in September 1934, and those from Colorado Springs and Pueblo, Colo., were received in October 1934. Assistant Chemist C. G. Remsburg carried out the determinations other than fluoride and boron, using mostly the methods given in the Standard Methods of Water Analysis of the American Public Health Association. The boron determinations were made essentially by the method of Foote (*7*).

school on the day of the examination were found to have had an unbroken history of residence and constant use of the city water supply.

As a result of the house-to-house recheck, the mottled enamel incidence increased, however, only 6.2 percent in Monmouth, 3.4 percent in Galesburg, 1.6 percent in Pueblo, and decreased 0.5 percent in Colorado Springs. For the purpose of computing an approximate mottled enamel index of a community it would appear on the basis of these examinations that, while it is necessary to obtain accurate water histories by careful questioning, a house-to-house recheck may be omitted. The errors in the history as given by the child on individual questioning are apparently largely compensatory, not cumulative, and the effect on the figures representing the distribution of severity and incidence is comparatively small.

As was indicated in the introduction, the study undertaken included six cities where mottled enamel was known to be endemic, and five other cities with comparable conditions were included as "controls." The latter were considered as controls because, on the basis of available reports, they were apparently free of mottled enamel. Monmouth was one of the six cities which had been reported as having mottled enamel while Galesburg, because it was reported as apparently free of mottled enamel, was chosen as the "control" for Monmouth. But, as already indicated above, a careful survey shows that Galesburg does not differ materially from Monmouth, and both may be given the same mottled enamel index.

A cursory examination of the fluctuating fluoride content of the Colorado Springs municipal water indicates why correlation of clinical observations with a single fluoride determination should be made with extreme caution. In this instance, if the field study had been made in August the index based on the clinical observations would have been correlated with a fluoride content of 1.8 p.p.m. of fluoride, while if the same survey had been made in February, March, or April, the index would have been correlated with a fluoride content of 3.0 p.p.m.

In accordance with previously described means (*8*) of determining the mottled enamel index of a community, the application of this method to the percentage distribution of severity as listed in tables 2 and 3 indicates that the mottled enamel index of Colorado Springs, Monmouth, and Galesburg is "slight," and that of Pueblo is "negative."

ACKNOWLEDGMENTS

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SUMMARY

1. The "mottled enamel index" of Colorado Springs, Colo., is "slight." A milder type of mottled enamel is endemic in Monmouth and Galesburg, Ill.; the mottled enamel index of both communities is likewise "slight." In Pueblo, Colo., the index is "negative."

2. The mean annual fluoride (F) content, based on monthly examinations, of the municipal water of Colorado Springs, Colo., between November 1933 and October 1934 was close to 2.5 parts per million. The corresponding mean annual fluoride content of the municipal waters of Monmouth, Ill., Galesburg, Ill., and Pueblo, Colo., was close to 1.7, 1.8, and 0.6 parts per million, respectively.

3. In 4 groups of 9-year-old school children, numbering 142, 187, 215, and 253, respectively, in communities of a fairly stable population, only about 20 percent (minimum, 20.4; maximum, 22.8) were found to have a history of continuous residence and constant use of the municipal water supply from birth.

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SOME EPIDEMIOLOGICAL ASPECTS OF CHRONIC ENDEMIC DENTAL FLUOROSIS

H. Trendley Dean and Elias Elvove

The endemic hypoplasia of the permanent teeth known in this country as mottled enamel was first reported by Eager (*1*) in 1901. The first investigation in the United States was the extensive one of Black (*2*), and McKay (*3*), published in 1916. At present in this country alone there are more than 200 areas where endemic mottled enamel has been confirmed by survey; in addition there are approximately 100 areas where the endemicity has been reported but not confirmed by survey. These approximate 300 areas are distributed among 23 different states.

Table I.—*Fluoride* (*F*) *content of monthly samples*

(Parts per million)

Month and Year	*Lubbock Texas*	*Amarillo Texas*	*Conway South Carolina*	*Plainview Texas*	*Mullins South Carolina*	*Big Spring Texas*
November, 1933		2.4	4.0		0.9	
December		2.5	3.9		0.9	
January, 1934	4.3	2.5	4.0	2.7	0.9	0.3
February	4.3	2.5	4.1	3.0	1.0	0.3
March	4.0	2.4	4.1	2.8	0.9	0.3
April	4.3	5.0	4.1	2.9	0.9	0.3
May	4.3	5.3	4.0	2.8	1.0	0.7
June	4.4	5.3	4.1	2.9	0.9	0.9
July	4.5	5.6	4.0	3.0	0.8	0.9
August	4.4	5.6	3.9	2.8	0.8	1.0
September	4.7	2.7*	4.1	2.9	0.9	1.1
October	4.4	5.0*	4.1	2.9	1.0	1.0
November	4.2			2.9		1.1
December	4.5			2.8		1.0
Mean annual fluoride content	4.36	3.9	4.03	2.87	0.9	0.7

* The Amarillo municipal water is obtained from two sources: October to March, inclusive, each year from the McDonald wells. April to September, inclusive, each year from the Palo Duro. In September, 1934, the city water was obtained from the McDonald supply on account of a break-down in the lines of the Palo Duro supply. In October, 1934, the water used in the city was from the Palo Duro; in November, the supply was again obtained from the McDonald wells.

The incidence of affection in an endemic area is relatively high, often 90 per cent, and in some instances all children exposed throughout the period of calcification of the teeth are affected. In the light of present knowledge, mottled enamel is a water-borne disease associated with the ingestion of toxic amounts of fluoride present in the water used for drinking and cooking during the period of tooth calcification. The minimal threshold has not yet been definitely established but studies to date would suggest that amounts below 1 p.p.m., expressed in terms of fluorine (F), are of no public health significance.

There is apparently no race, color, or sex differential. With respect to the teeth, the causative factor is operative only during the period of calcification, which, with the exception of the third molar represents approximately the first decade of life. There is some indication (*4*) that the skeletal system might likewise be affected; if this is true, it would be necessary to extend the time range to include adults.

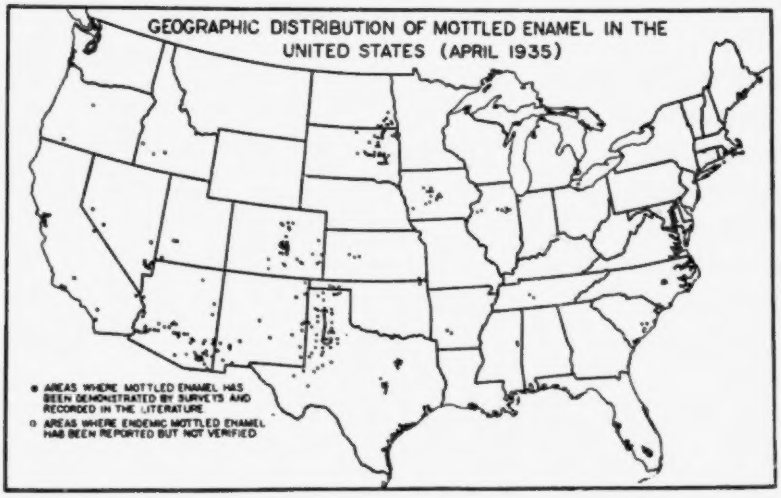
GEOGRAPHIC DISTRIBUTION OF MOTTLED ENAMEL IN THE UNITED STATES (APRIL 1935)
● AREAS WHERE MOTTLED ENAMEL HAS BEEN DEMONSTRATED BY SURVEYS AND RECORDED IN THE LITERATURE
○ AREAS WHERE ENDEMIC MOTTLED ENAMEL HAS BEEN REPORTED BUT NOT VERIFIED

Figure I

The macroscopic pathology in the human has been classified according to severity thus: normal, questionable, very mild, mild, moderate, moderately severe, and severe. These various gradations have previously been described (*5*). This classification, depending on the degree of severity of the affection, permitted in turn the development of the following "mottled enamel indexes" of a community (*6, 7*): negative, border-line, slight, medium, rather marked, marked, and very marked. Several detailed studies (*8, 9, 10*) concerning the histopathology of this disease have been reported. An analogous hypoplasia developed under natural conditions has been observed in certain domestic animals (*11, 12*). The sole hope in the solution of this problem lies in prevention. Since the ameloblasts cease functioning at the time of eruption of a tooth, such ameliorative treatment as is possible is naturally very limited in scope and generally unsuccessful. Bierring (*13*) in referring to this disease recently has recorded that "although different in nature from typhoid fever, [it] represents a permanent physical defect of distressing character."

Mottled enamel has direct relationships to other forms of oral pathology, some of these relationships being at times paradoxical. In spite of their defective structure, mottled enamel teeth, according to McKay (*14*), exhibit no greater liability to caries than do normally calcified teeth, an inference apparently sustained by the investigations of Masaki (*15*), Ainsworth (*9*), and Erausquin (*16*). Masaki (*15*) and Ainsworth (*9*), in addition, maintain that the eruption of the secondary dentition is delayed.

From observations made by one of us (HTD) in areas of relatively high fluoride concentration (more than 4.0 p.p.m.)

there is, likewise, sufficient evidence to suggest an apparent tendency to a higher incidence of gingivitis (fig. I).

POSSIBILITY OF ERROR IN NON-QUANTITATIVE STUDIES

A review of the literature indicates that surveys of the past have been largely non-quantitative. In many instances data of little value are submitted relative to two important conditions, namely, (a) the number of children in the group studied, together with the length of time of their constant exposure, and, (b) possible changes in the chemical composition of the water used during the life period of the group examined.

The number in the group examined should consist of at least 25 children 9 years of age or older who had used the water under investigation continuously since birth for both drinking and cooking. Breaks in continuity totaling less than 30 days in any one calendar year are excepted. In instances where the examination of the first 25 children discloses an incidence of less than 75 percent, the number in the group examined should be increased to at least 50, if possible. In other words, when more than 25 per cent are diagnosed as "normal" or "questionable," the size of the group examined should be increased to compensate for fluctuations in sampling and their possible effect on the computation of the community mottled enamel index.

TABLE II.—*Analyses of the waters* used*

(PARTS PER MILLION)

	Lubbock Texas	*Amarillo Texas*	*Conway South Carolina*	*Plainview Texas*	*Mullins South Carolina*	*Big Spring Texas*
Residue on evaporation	882.0	468.0	633.1	390.0	172.0	314.0
Loss on ignition	77.0	73.7	16.6	37.0	18.2	27.0
Fixed residue	805.0	394.3	616.5	353.0	153.8	287.0
Silica (SiO_2)	64.0	51.8	17.0	62.0	43.0	19.0
Iron (Fe)	0.1	0.08	0.06	0.1	0.08	0.03
Aluminum (Al)	0.2	0.3	0.06	0.3	0.25	0.50
Calcium (Ca)	60.7	43.9	2.3	48.5	1.3	73.2
Magnesium (Mg)	72.6	47.8	0.5	35.8	0.8	6.6
Sodium and Potassium (calculated as Na)	131.4	54.1	255.1	41.9	49.3	31.1
Carbonate (CO_3)			36.0			
Bicarbonate (HCO_3)	342.8	389.2	256.0	331.8	124.2	226.9
Sulphate (SO_4)	255.0	52.0	3.4	32.5	4.3	31.9
Nitrate (NO_3)	1.0	4.4	1.0	2.2	0	4.0
Chloride (Cl)	111.8	13.5	49.5	31.8	7.9	34.0
Fluoride (F)	4.2	5.6	4.1	2.9	1.0	1.1
Phosphate (PO_4)	0	0	0	0	7.1	0
Boron (B)	0.5	0	2.5	0	0	0

* These samples of water from Lubbock, Plainview, and Big Spring were received in November, 1934; the sample from Amarillo, in August, 1934; and those from Conway and Mullins, in October, 1934. Assistant Chemist C. G. Remsburg carried out the determinations other than fluoride and boron, using mostly the methods given in the *Standard Methods of Water Analysis* of the American Public Health Association. The boron determinations were made essentially by the method of Foote.[19]

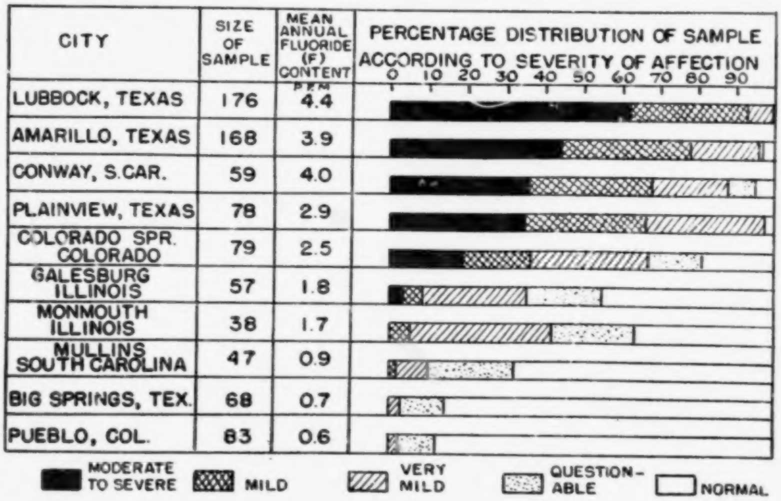

FIGURE II.—*Severity of mottled enamel in children of 10 selected cities and the mean annual fluoride (F) content of the municipal water supply 1933–34.*

Studies of the past have, in most cases, failed to take into consideration what has recently been revealed by carefully conducted surveys which included the individual water histories of each child rechecked by an interview with the child's parent. These surveys (7) have shown, at least with respect to the 9-year-old group in the four cities studied, that only about 20 percent used the city water continuously from birth.

The need for an adequate clinical sample is obvious when we consider that the causative factor of mottled enamel is probably operative during the entire period of calcification of the permanent teeth; and an observed effect may be the result of a comparatively low fluoride concentration operating during the entire period, or a relatively high concentration for a shorter period. In endemic areas where the domestic water contains a fluoride concentration only slightly above the minimal threshold, the production of visible signs of mottled enamel in the susceptibles of the group examined would probably depend upon the continuous use of the water during the entire period of calcification. On the other hand, in areas where the domestic water contains a comparatively high fluoride concentration, its use during a part of the period of calcification is sufficient to produce observable mottled enamel. A careful analysis of the data from the Bauxite survey (*17*) makes this latter point apparent.

Because of possible changes in the fluoride content of water supplies, it is obvious that an attempt to correlate clinical observations with a single fluoride determination of a municipal water associated with endemic mottled enamel introduces the possibility of questionable correlation. In respect to water from deep wells, the fact that the mineral content usually varies within comparatively narrow limits might be misleading until it has been definitely ascertained that there have been no changes in the physical setup of the water supply during the lifetime of the children. Hence the amount of fluoride in a water sample taken at the time of the clinical examination may mean little unless a complete history of the water supply concomitant with the life of the children examined, has been obtained.

It must also be borne in mind that some municipalities using deep wells for their common water supply have more than one source of deep well water. For instance, the largest city in the United States known to be affected with mottled enamel regularly uses water from one group of wells for 6 months of the year, and from an entirely different group for the other 6 months. This rotation has been followed for the past 4 years, and since one supply has about twice as much fluoride as the other, it is evident that a single sample representing only one of these supplies would be valueless in correlating chemical findings with clinical observations. Moreover, the addition of new wells, or the abandonment of old ones during the lifetime of the group examined may introduce variable factors that would make it impossible to draw reliable conclusions from the chemical findings disclosed at the time of the examination. Furthermore, in dealing with surface waters or shallow wells, the seasonal and annual rainfall or other meteorological conditions become factors of importance. In this study, therefore, consecutive monthly water samples were obtained for 1 year and a fluoride determination was made on each sample.

PRESENT STUDY

This paper presents a comparison of the results obtained in the investigation of the following 10 cities: Monmouth and Galesburg, Ill.; Colorado Springs and Pueblo, Colo.; Lubbock, Amarillo, Plainview, and Big Spring, Tex.; and Conway and Mullins, S.C.

The detailed results, including chemical analyses, noted in the study of the Illinois and Colorado areas have been reported (*7*). An approximate mottled enamel index of each of the 4 Texas cities has already been reported (*6*), but the chemical data are included in the present paper. Furthermore, there are included in this report, for comparative purposes, chemical findings of and certain relevant clinical observations from a recently made study in South Carolina.

The fluoride content of the monthly samples of water was estimated colorimetrically by means of the zirconium-alizarin reagent (*18*). The results obtained are given in table I.

As has been pointed out previously (*7*), it may be reasonable to suppose that the mottled enamel index will be found to depend entirely on the fluoride concentration of the drinking water; but it is possible, on the other hand, that other constituents of the water may have some influence on the activity of the fluoride. For this reason it appears desirable that a careful survey of a community for chronic endemic dental fluorosis should include also, for the present at least, a chemical analysis of the drinking water for constituents other than fluoride. Results of the chemical analyses of the waters from the South Carolina and Texas cities are given in table II.

The water histories, especially with respect to variation, and other comparative data obtained in the study of the 10 cities referred to are given in table III.

DISCUSSION

An analysis of the history of the common water supply respecting continuity clearly indicates the extreme caution necessary in attempting to correlate clinical observations including verified water biographies, with a single fluoride determination or even an annual arithmetical mean of the fluoride content when the communal water supply itself contains one or more extrinsic variables. In more than half of the cities in the group there are sufficient interruptions to warrant caution in attempting to correlate clinical observations with the composition

TABLE III.—*Water histories and other comparative data of the cities studied* *

City and State	Population (1930)	Description of Water Supply with Respect to Variation	Quantitative Evaluation		Clinical				Mottled enamel index of the community	Mean annual fluoride (F) content (1933-1934) (parts per million)
			Presence or absence of requisites	Classification	Age group or school grade examined	Number of children examined who stated they had used municipal water continuously	Number showing mottled enamel	Incidence		
Colorado Springs, Colo.	33,237	Surface supply; melted snow from Pike's Peak stored in 7 mountain reservoirs 9,300′ to 12,000′ in altitude. No changes in present supply during past 20 years.	Present	A	9 year old	79	53	67.0	slight	2.5
Monmouth, Ill.	8,666	Since 1925 from two 2,445′ wells drilled to the "Potsdam" stratum of the Cambrian sandstone. Upper water-bearing strata cased off.	Present	A	9 year old	38	16	42.1	slight	1.7
Mullins, S. C.	3,158	Municipal water supply obtained from 2 deep wells, 350′ and 360′ deep and drilled in 1913 and 1921, respectively.	Present	A	9, 10, 11 years old	47	5	10.6	negative-border line	0.9
Pueblo, Colo.	50,096	Surface supply; Arkansas River. Samples taken from North Pueblo supply; clinical examinations likewise limited to children in northern half of city. North Pueblo supply from same source during past 46 years.	Present	A	9 year old	83	2	2.4	negative	0.6
Lubbock, Tex.	20,520	Municipal supply obtained from 8 wells, #1 (1925), #2 (1917), #3 and #4 (1924), #5 (1928), #6 (1930), #7 (1931), and #8 (1934). City water in general is a composite of these various wells distributed from 5 reservoirs in which water from specific wells is impounded. No. 4 well furnishes about 25% of the supply; Nos. 1, 2, 5, and 6 about 15% each, and Nos. 3, 7, and 8 about 5% each. Depth ranges from 140′ to 150′; all wells have perforated casings through each water bearing stratum. About half of the time only 2 pumps are operated and one section of the city may be served by one or two wells and another section by another well or group of wells.	Absent	B	4th 5th 6th grades	176	174	98.8	marked	4.4
Amarillo, Tex.	43,132	Prior 1927 from 35 wells, 250′ deep, located in various parts of city. In 1927 Palo Duro supply, 20 mi. SW of city consisting of 10 wells, 180′ deep, installed, and used exclusively until 1931 when McDonald supply consisting of 5 280′ wells, 7 mi. SW of city was installed. Since 1931 municipal supply from two sources: Oct.-March inclusive each year from McDonald wells; April-Sept. inclusive each year from the Palo Duro.	Absent	B	4th 5th 6th grades	168	162	96.4	rather marked	3.9

* Clinical examinations at Monmouth and Galesburg, Ill., were made during October, 1934; those of the 4 Texas cities during November, 1934, Colorado Springs and Pueblo, Colo., during April, 1935, and Mullins and Conway, S. C., during September, 1935.

Table III.—*Water histories and other comparative data of the cities studied**—Con.

City and State	Population (1930)	Description of Water Supply with Respect to Variation	Quantitative Evaluation		Clinical				Mottled enamel index of the community	Mean annual fluoride (F) content (1933–1934) (parts per million)
			Presence or absence of requisites	Classification	Age group or school grade examined	Number of children examined who stated they had used municipal water continuously	Number showing mottled enamel	Incidence		
Conway, S. C.	3,011	Municipal water supply is obtained from 3 artesian wells, a 3½″ well drilled in 1896, 450′ deep; a 10″ well drilled in 1918, 400′ deep, and a 3″ well drilled in 1927, 305′ deep. All wells cased entire depth. Casings of the 400′ and 450′ wells have "slots" at the higher water bearing levels and water from these 2 wells represents a composite of more than one water bearing stratum. About 85% of the city water is obtained from the 10″ well; the remainder from the 305′ well. Well drilled in 1896 supplies negligible amount. Analysis of a sample from each of the 3 wells shows slight variation in the fluoride (F) content; approximately 0.2 p.p.m.	Absent	B	9, 10, 11 years old	59	52	88.1	rather marked	4.0
Plainview, Tex.	8,834	Prior to 1926 from 2 wells, 95′ and 175′ deep (old supply); 1926–1928 from one well 275′ deep (new supply). From 1928 to date supply is a composite one, 75% from "new" supply and 25% from "old" supply. All wells within city limits are "gravel backed" thus permitting water from different strata to mix.	Absent	B	5th 6th 7th 8th grades	78 †	76 †	97.4	rather marked	2.9
Galesburg, Ill.	28,830	Since 1928 from 2 2,414′ wells drilled to the "Potsdam" stratum of the Cambrian sandstone. During 1924–1928, 60% of municipal supply from first 2,414′ well and 40% from 2 wells in St. Peters sandstone, 1,245′ and 1,252′ deep.	Absent	B	9 year old	57	20	35.1	slight	1.8
Big Spring, Tex.	13,735	Municipal supply obtained from 23 wells. One-third of supply from 13 wells, 260′ deep drilled in 1923. Two-thirds of supply from 8 wells, 280–300′ deep, drilled in 1927. During 1894–1923 all municipal water from "Old Park" supply, which was supplemented until 1927 by 1923 group of wells. During 1927–1933, "Old Park" supply shut down. Between Nov., 1933, and May, 1934, 1927 group of wells also shut down. Wells located 2, 6, and 9 miles, respectively, south of Big Spring.	Absent	B	5th 6th grades	68	2	2.9	negative	0.7

* Clinical examinations at Monmouth and Galesburg, Ill., were made during October, 1934; those of the 4 Texas cities during November, 1934, Colorado Springs and Pueblo, Colo., during April, 1935, and Mullins and Conway, S. C., during September, 1935.

† Large part of 1934 sample calcified their teeth while using "old" water supply, the "F" content of which is unknown. Recent survey (March, 1936) shows incidence of 87.6 and index of "medium."

of the water at the time of the clinical survey.

The application of epidemiological principles for the purpose of determining a minimal threshold of toxicity produced under natural conditions presupposes that inferences shall not be drawn from studies of cities in which there is an interfering relevant variable in one or both of the factors which are to be correlated. The magnitude of the population of these 10 cities makes possible a clinical examination of a sufficiently large sample in each community having the essential characteristic of continuously using a common water supply for domestic purposes. But when an attempt is made to correlate the mottled enamel index of the community with the mean annual fluoride content, only four of the cities are appropriate for quantitative evaluation; in the others, an approximate evaluation only is possible.

With respect to an adequate clinical sample, it has been found desirable, if not necessary, in instances where the examination of the first 25 children discloses an incidence of less than 75 percent, to increase the minimal number of the group, whenever possible, to at least 50. In other words, when more than 25 percent are diagnosed "normal" or "questionable," the increase in the number examined naturally tends to compensate for the fluctuation in sampling and its corresponding influence on the computation of the community mottled enamel index.

The data obtained in the study of these 10 cities and the fact that many reports in the literature generally omit inherent basic data required for epidemiological purposes suggest the classification of endemic areas under three heads:

Class A—Communities where the requisites for a quantitative evaluation are present and have been analyzed

Class B—Communities where changes in the source of water supply and/or population preclude quantitative evaluation. In some instances, however, a careful analysis of the variable factor or factors may suggest an approximate evaluation

Class C—Communities which have been reported in the literature as affected with chronic endemic dental fluorosis (mottled enamel) but where the requisites for a quantitative evaluation were not stated.

In this study, Colorado Spring, Colo.; Monmouth, Ill.; Mullins, S.C., and Pueblo, Colo., are illustrative of Class A. Lubbock and Amarillo, Tex.; Conway, S.C.; Plainview, Tex.; Galesburg, Ill., and Big Spring, Tex., would come under Class B. A high percentage of endemic areas previously reported in the literature would be classified under C.

SUMMARY

1. The requisites for a quantitative evaluation of an area showing chronic endemic dental fluorosis (mottled enamel) are: (a) a common water supply whose history discloses no relevant changes in either its physical setup or its source during the period concomitant with the life of the group examined; and (b) clinical examinations, and observations recorded quantitatively, of a minimum of 25 children, 9 years of age or over, who have used a common water supply continuously since birth for both drinking and cooking. Breaks in continuity totaling less than 30 days in any one calendar year are excepted. Moreover, in instances where the examination of the first 25 children discloses an incidence of less than 75 percent, an attempt should be made to increase the

number of children to 50 or more in order to compensate for fluctuation in sampling.

2. For the purpose of quantitative study, affected communities are classified on the basis of the presence or absence of the requisites for quantitative evaluation, as follows: Class A includes communities where the requisites for a quantitative evaluation are present and have been analyzed; Class B includes those where changes in source of water supplies and/or population make impossible the determination of a quantitative evaluation; and Class C includes communities which have been reported as affected with mottled enamel without stating whether the requisites for a quantitative evaluation are present or absent.

3. The percentage distribution of severity permits the computation of an actual or approximate "mottled enamel index" of an affected area. This index in turn may be correlated with the fluoride content of the water supply, expressed as an arithmetical mean of 12 consecutive monthly determinations.

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CHRONIC ENDEMIC DENTAL FLUOROSIS (Mottled Enamel)

H. Trendley Dean *

The endemic hypoplasia of the permanent teeth known as chronic endemic dental fluorosis, or mottled enamel, is a water borne disease associated with the ingestion of toxic amounts of fluorides in the water used for cooking and drinking during the period of calcification of the affected teeth. The permanent teeth in particular are affected, although in areas of medium to marked severity the signs of mottled enamel are at times observable on certain of the deciduous teeth.

The causative factor of mottled enamel is operative during the period of tooth development. Hence the affected teeth erupt showing the characteristic markings of hypoplasia. Normally calcified teeth erupt showing a smooth, glossy, translucent structure, usually of a pale creamy white color. Teeth affected with mottled enamel, on the contrary, erupt showing a dull, chalky white appearance which in many instances later take on a characteristic brown stain, the frequency of brown stain increasing with age. In areas of marked severity, the surface of the teeth may in addition be marked by discrete or even confluent pitting. As the enamel forming organisms, the ameloblasts (ganoblasts), cease functioning at the time of the eruption of a tooth, mottled enamel is a permanent physical disfigurement. For purposes of classification, the various types (*1*) of the several degrees of severity have been divided into normal, questionable, very mild, mild, moderate, moderately severe, and severe.

References to this disease in the literature are comparatively recent, the first report being that of Eager (*2*) in 1901. The extensive investigations of McKay (*3*) published in 1916 pointed unmistakably to mottled enamel being a water borne disease. Final proof of the validity of this hypothesis was furnished by McKay (*4*) with the successful consummation of the Oakley (Idaho) experiment.

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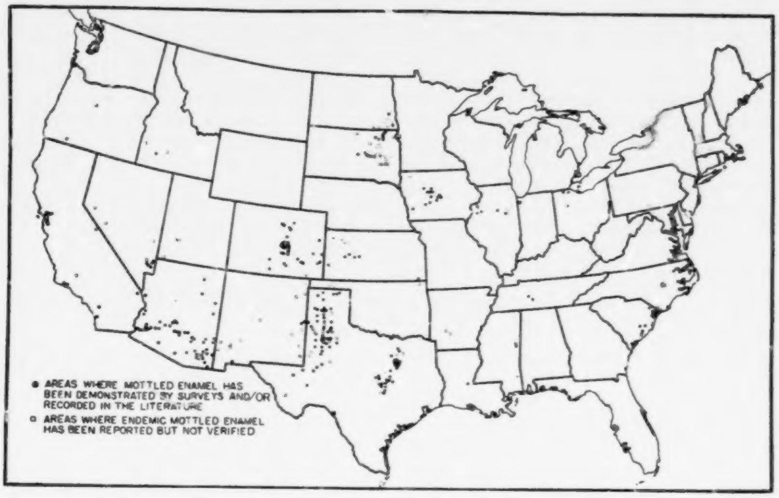
Shortly after the publication of McKay's original report in 1916 the significance of this endemic defect of the teeth as a health problem was inferred by Smith (*5*), who late in the same year called the attention of public health workers to the importance of mottled enamel as a hygienic question.



● AREAS WHERE MOTTLED ENAMEL HAS BEEN DEMONSTRATED BY SURVEYS AND/OR RECORDED IN THE LITERATURE
○ AREAS WHERE ENDEMIC MOTTLED ENAMEL HAS BEEN REPORTED BUT NOT VERIFIED

FIGURE 1.—*Geographic distribution of mottled enamel in the United States in April 1936.*

PREVALENCE

The distribution is world wide. In the United States there are about 335 endemic areas distributed among 25 states. Eighty-six percent of these areas are located west of the Mississippi River, the most severely affected state being Texas.

In that portion of West Texas studied, a region about equal in square miles to the state of Pennsylvania, the causative factor of mottled enamel is operative over a vast district. A high percentage of the many thousands of children residing in this region during the period of calcification of the permanent teeth have developed, or are developing, mottled enamel. The ubiquity of the disease there is startling. Of the approximately 335 surveyed or reported endemic areas in the United States, 94, or about 28 percent, are found in Texas. Other states in which mottled enamel is known to constitute an important public health problem are Colorado South Dakota and Arizona. East of the Allegheny Mountains a number of small communities located in the Atlantic coastal plain region of Virginia, North Carolina and South Carolina are affected.

Among the foreign countries the Argentine Republic is no doubt the most seriously affected, with about 175 endemic areas reported (*6*). Other countries where endemic areas have been recorded are England (*7*), Italy (*8*), North Africa (Morocco, Tunisia and Algiers) (*9*), China (*10*) and Japan (*11*). Extensive studies are apparently being carried on at present in the Argentine Republic and North Africa. Other countries in which this problem has been the subject of investigation within the past few years are England, Italy and Japan.

ETIOLOGY

There is strong presumptive evidence that the causative factor of mottled enamel is the presence of toxic amounts of fluorine, present as a fluoride, in the water used for drinking and cooking during the period of calcification of the permanent teeth. The conclusions in 1931 of three independent investigations (*12*) pointed to fluorine as the etiologic factor. A carefully controlled experiment (*13*) in which municipal water was used from Conway, S.C., an endemic area, demonstrated that changes in the teeth of white rats given a concentrate of the Conway water were similar to those produced by water containing comparable amounts of sodium fluoride. The extensive survey about this time by Boissevain (*14*) in Colorado added further evidence to support this theory. Further experiments at this laboratory indicated (*15*), at least with respect to white rats, that, while fluorine is the chief factor, other conditions possibly influence its action. There was a marked difference in the effect of a given quantity of sodium fluoride, depending on whether it was administered in the water or in the food.

Thorough surveys thus far made, though limited in number, have indicated that when an adequate number (*16*) of children are examined in a community having the requisites for quantitative evaluation there is an orderly uniformity in the group response to the fluoride concentration of the communal water supply, with regard both to the incidence and to the percentage distribution of severity, particularly the latter.

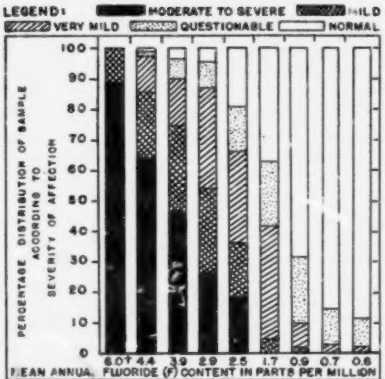
The minimal threshold of toxicity in drinking water has not yet been definitely established, but studies to date would suggest that amounts not exceeding one part per million, expressed in terms of fluorine (F), are of no public health significance.

PEOPLE AND TIME FACTOR

There is apparently no race, color, or sex differentiation. In endemic areas—at least with respect to the permanent teeth—only those individuals are affected who have used a water containing toxic amounts of fluorides during the period of calcification of their permanent teeth. Whether or not there are other signs or symptoms of fluorosis as yet unobserved among the adult population of an endemic area is at present undetermined. The work of Boissevain and Drea (*17*) on human bones, taken in conjunction with the reports of various workers in comparative pathology (*18*), is at least suggestive of skeletal involvement. If so, the time factor must be extended to cover adults. Lemmon (*19*), a pediatrician of Amarillo, Texas, an endemic area, records that "some of these babies have more tendency to bowing of the legs, even in the face of constant antirachitic therapy, thus supporting the theory that the toxic fluorides interfere with bone and dental metabolism."

HISTOPATHOLOGY

The first report on the pathologic histology of this disease was that of Black (*20*) in 1916, who noted that the identifying characteristic was the absence of the cementing, or interprismatic substance, between the outer fourth and the outer third of the enamel rods. Black states that no injury to the enamel rod was observed and that the dentin was normal. In 1923 Williams (*21*) confirmed the observations of Black respecting the absence or gross malformations of the interprismatic substance and added that in some instances the defective enamel structure extends to the dentoenamel



MEAN ANNUAL FLUORIDE (F) CONTENT IN PARTS PER MILLION	6.0†	4.4	3.9	2.9	2.5	1.7	0.9	0.7	0.6
NO. CHILDREN EXAMINED *	38	189	289	97	79	38	47	66	63
AGE GROUP OR SCHOOL GRADE EXAMINED	4th-5th AND 6th GRADE	9-10 11-12 YEAR OLD	9-10 11-12 YEAR OLD	9-10 11-12 YEAR OLD	9 YEAR OLD	9 YEAR OLD	9-10 & 11 YEAR OLD	5th AND 6th GRADE	9 YEAR OLD
YEAR EXAMINED	1934	1936	1936	1936	1935	1934	1935	1934	1935
CITY AND STATE	POST, TEXAS.	LUBBOCK, TEXAS.	AMARILLO, TEXAS.	PLAINVIEW, TEXAS.	COLORADO SPRINGS, COLORADO.	MONMOUTH, ILLINOIS.	MULLINS, S. CAROLINA.	BIG SPRING, TEXAS.	PUEBLO, COLORADO.

† SERIES OF MONTHLY SAMPLES NOT COMPLETED; PRELIMINARY ESTIMATION BY SENIOR CHEMIST E. ELVOVE, U.S.P.H.S.

* ALL CHILDREN EXAMINED STATED THEY WERE BORN IN THE COMMUNITY AND HAD USED MUNICIPAL WATER CONTINUOUSLY THROUGHOUT LIFE.

FIGURE 2.—*Severity of mottled enamel in children of nine selected cities and the mean annual fluoride (F) content of the municipal water supply in 1933–1934. (All fluoride determinations were made by senior chemist Elias Elvove, United States Public Health Service, and, with the exception of those for Post, Tex., have been reported in two previous articles [Dean and Elvove, footnotes 16 and 28]. For information concerning the history of each water supply, seasonal variations or chemical methodology, the reader is referred to these papers.)*

junction. Black's opinion that the defect in mottled enamel was limited to the substance between the rods was based on examinations under the low and medium powers of the microscope and without the benefit of the silver nitrate staining technique as used by Williams. The latter, using a high resolving power of the 2 mm. and 3 mm. apochromatic objectives, found that the enamel rods were incompletely calcified. Imperfectly fused granules and small spherical bodies were frequently observed, the larger of these globular masses appearing to be identical with what are known as calcospherites. The histologic picture of mottled enamel disclosed no essential difference from that of incompletely calcified forming enamel, or enamel showing the early stages of caries.

In 1925 Beust (*22*) called attention to the fact that, in addition to the enamel, the dentin was likewise affected, a condition which he termed mottled dentin. Black had stated that the dentin was normal, while no reference to this tissue had been made by Williams. The observation of Beust with respect to dentinal disorder has been confirmed by Ainsworth (*7a*), who reported imperfectly calcified dentin with interglobular spaces such as may be seen in any ordinary case of hypoplasia. Ainsworth further notes that the pitting observed macroscopically on the surface of the enamel may be explained as a breaking off of the ends of enamel layers weakened by the loss of the interprismatic substance. He adds that the layers of enamel formed between the striae of Retzius are broken off almost at right angles to the striae, so that they show on the floor of the pits as a series of angular outcrops, giving in one section a definitely serrated appearance not unlike the pits in other forms of hypoplastic teeth. Erausquin (*23*) reports that the permeability of mottled enamel is comparable to that of immature normal (unerupted) enamel and enamel affected by caries. The permeable zone that characterizes mottled enamel is always the most external; its intensity diminishes from the outside inward.

RELATION TO OTHER ORAL PATHOLOGIC CHANGES

In spite of its defective structure, mottled enamel teeth, according to McKay (*24*), exhibit no greater liability to caries than do normally calcified teeth, an inference apparently substantiated by the studies of Masaki (*11*), Ainsworth (*7a*) and Erausquin (*6*). Masaki (*11*) and Ainsworth (*7a*) have likewise called attention to an apparent delay in the eruption of permanent teeth of children residing in endemic areas, an observation which I have as yet been unable to confirm. Ainsworth (*7a*) and Lemmon (*19*) have also suggested that the deciduous teeth erupt somewhat later than usual.

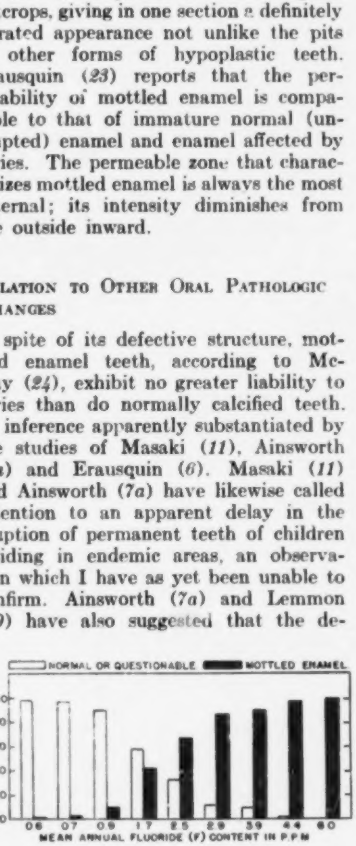


FIGURE 3.—*Graphic summary of data in figure 2, showing quantitative relation of fluoride (F) concentration of clinical effect.*

From observations that I made in areas of relatively high fluoride concentration (more than four parts per million of fluorine) there is sufficient evidence to suggest that there is an apparent tendency toward a higher incidence of gingivitis.

DOMESTIC ANIMALS

The production of an analogous pathologic condition under natural conditions in certain domestic animals has been reported in North Africa (*9*), the United States (*25*) and the Argentine Republic (*23*). This endemic hypoplasia particularly affects those domestic animals requiring several years for the calcification of their permanent teeth, such as the horse, the cow and the sheep. In North Africa, especially, this phase of the problem has been the subject of considerable investigation (*26*).

INCIDENCE

The incidence in an endemic area may be high. Among those children exposed to waters containing relatively high amounts of toxic fluorides during the first 8 years of life, an incidence of from 80 to 90 percent is not uncommonly observed. In some instances it may reach 100 percent. Both the incidence and the percentage distribution of severity of the condition has been found to vary in relation to the fluoride concentration of the water. For epidemiologic purposes and subsequent correlation with chemical and other studies, the determination of the community mottled enamel index is advisable. These indexes, which have previously been described (*1b*), are negative, borderline, slight, medium, rather marked, marked, and very marked.

The actual mottled enamel index of a community should not be computed unless there have been no physical changes in the set up of the water supply concomitant with the life period of the group examined. For practical purposes, however, an approximate mottled enamel index may be developed if the interruptive variable in the water supply is such that it can be mathematically appraised. The group of children must consist of at least 25 individuals 9 years of age or older, whose time of risk of exposure has been constant, meaning that the children were born in the community, had lived there all their lives (short breaks in continuity totaling less than 30 days in any calendar year excepted) and had always used the municipal or communal water supply both for cooking and for drinking. In cases in which the examination of the first 25 children discloses an incidence of less than 75 percent, it has been found desirable, if not necessary, to increase the number in the group to 50 or more in order to compensate for fluctuations in sampling and their possible effect on the computation of the mottled enamel index.

The group examined should preferably consist of 9, 10, 11 and 12-year-old children. Clinical observations limited to 9-year-old children in areas in which the fluoride concentration of the water is less than 3 parts per million have a tendency to give a slightly lower incidence than if the survey had embraced the four age groups mentioned. Two related factors are probably the cause of this somewhat lower incidence in a survey limited to the 9-year-old group. First, in endemic areas of relatively low fluoride concentration (less than 2 parts per million) there is, in a fair proportion of the children of comparable and constant residence and water history, a tendency to show the milder forms of mottled enamel only on the bicuspids and the second molars, a group of teeth which, according to Kronfeld (Development and Calcification of the Human Deciduous and Permanent Dentition, The Bur, March 1935) begin their calcification at a somewhat later date than the incisor-first molar group. This manifestation of mild dental fluorosis in teeth calcified at a later date is suggestive of a cumulative action of fluorine. Second, based on an analysis of the 162 schedules of the Colorado Springs-Pueblo survey (*16*), only about 1 percent of the permanent second molars, 7.2 percent of the second bicuspids and 20.5 percent of the first bicuspids were erupted in the 9 year age group. It follows, therefore, that certain 9-year-old children are necessarily classified as normal on the basis of the absence of mottled enamel on the incisor-first molar group when, if the same individual were examined a year or two later, it might show objective signs of mottled enamel on the bicuspid-second molar group and be so classified. Adjusting for this minus variation, for instance, in a survey of a community like Colorado Springs where the mean annual fluoride content of the city water is 2.5 parts per million of fluorine, an examination of 9, 10, 11 and 12-year-old children would probably result in raising the incidence from 67 percent for the 9-year-old group exclusively to a general rate of about 75 to 80 percent if the sample consisted of the four age groups mentioned. Because the percentage distribution of severity in the 9-year group examined in this city is so near that of the next higher index, it is probable that, in this instance, the community mottled enamel index would be raised from "slight" to "medium." In areas where the fluoride content of the water is between 1.5 and 2 parts per million, it is unlikely that this difference would have any effect other than a slight increase in the incidence. In areas of relatively high concentrations, the effects of this variation would, of course, be negligible.

QUANTITATIVE RELATION OF CONCENTRATION TO ACTION

In surveys made of cities having the requisites for quantitative evaluation (*27*) and even where these requisites are closely approximate, there is a definite quantitative relation between the fluoride concentration and the clinical effect. Although a prognosis with respect to any one individual is obviously unwarranted, it is felt that prognosis relative to the group response to waters of varying fluoride concentration may be tentatively made at this time. From the continuous use of water containing about 1 part per million, it is probable that the very mildest forms of mottled enamel may develop in about 10 percent of the group. In waters containing 1.7 or 1.8 parts per million, the incidence may be expected to rise to 40 or 50 percent, although the percentage distribution of severity would be largely of the "very mild" and "mild" types. At 2.5 parts per million an incidence of about 75 to 80 percent might be expected, with possibly 20 to 25 percent of all cases falling into the "moderate" or a severer type. A scattering few may show the "moderately severe" type.

At 4 parts per million the incidence is, in general, in the neighborhood of 90 percent, and as a rule 35 percent or more of the children are generally classified as "moderate" or worse. In concentrations of 6 parts per million or higher an incidence of 100 percent is not unusual. In other words, we are dealing with a low grade chronic fluorine poisoning of children and the action on the group roughly follows the general pharmacologic observations of Shackell (*28*) respecting the quantitative relation of concentration to effect.

PREVENTION

In the light of present knowledge, this disease is readily preventable. The logical approach to the solution of this problem is, of course, avoiding the use of water containing fluorides in excess of the permissible limit. In some instances this can be accomplished by simply changing to a readily available source in the same neighborhood that is free of toxic amounts of fluorides. Examples of this are the towns of Oakley, Idaho, previously referred to (*4*), and Bauxite, Ark. (*29*), which abandoned its deep wells and turned to the nearby Saline River as the source of its water supply.

Where such changes are not entirely feasible, it may be possible in some cases, especially where the water contains less than 2 parts per million, to dilute an otherwise satisfactory water supply with another water that will bring the final fluoride content down within the permissible limits. And while these changes are being made, it is well to bear in mind that the only portion of the population known to need protection is the group of children between birth and 8 years of age, inclusive. For this group it is possible in many cases to provide distilled or cistern water for drinking and cooking purposes during the susceptible period, the first 8 years of life.

Finally, in those areas in which a satisfactory water is not available, the ultimate solution in such cases would have to depend on a suitable method, economically feasible, of treating the existing water supply by chemical means for the removal of toxic amounts of fluorides.

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FURTHER STUDIES ON THE MINIMAL THRESHOLD OF CHRONIC ENDEMIC DENTAL FLUOROSIS

By H. TRENDLEY DEAN and ELIAS ELVOVE

Late in 1933 an investigation was begun to determine what constitutes a permissible amount of fluoride in a domestic water supply. A mean annual flouride content of the domestic water supply was determined for each of a group of 10 cities. Twelve consecutive monthly water samples were sent by each of these cities to the National Institute of Health for chemical analyses. The results of the chemical study revealed the following range in fluoride concentration, the mean annual fluoride content of each common water supply being expressed in parts per million of fluorine (F): Pueblo, Colo., 0.6; Big Spring, Tex., 0.7; Mullins, S.C., 0.9; Monmouth, Ill., 1.7; Galesburg, Ill., 1.8; Colorado Springs, Colo., 2.5; Plainview, Tex., 2.9; Amarillo, Tex., 3.9; Conway, S.C., 4.0; and Lubbock, Tex., 4.4. Clinical examinations were recorded in each of these cities and the results of the investigation were published in two reports (*1*, *2*).

A careful analysis of these and subsequent data (*3*) indicated a definite quantitative relationship between the fluoride concentration and the clinical manifestations of dental fluorosis. There was an orderly uniformity in the group response to the fluoride concentration of the communal water supply with regard both to the incidence and the percentage distribution of severity, particularly the latter. With this fact demonstrable, it seemed desirable to focus further attention on the minimal threshold of toxicity, or the maximum amount of fluoride permissible in a domestic water supply.

PRESENT STUDY

For this study, four additional communities were selected in the autumn of 1935 for detailed quantitative study. These cities were Clovis, N. Mex., Webster City, Iowa, East Moline, Ill., and Junction City, Kans. A report of the survey of these four cities, prefaced by a description of their common water supplies, follows:

DESCRIPTION OF THE COMMON WATER SUPPLIES

The description and data concerning these municipal water supplies were furnished by Messrs. A. J. Whiting, vice president, New Mexico Utilities Co., Clovis, N. Mex.; E. R. Compton, city manager, Webster City, Iowa; Arnold Neihaus, East Moline, Ill., and Arthur Rathert, water superintendent, Junction City, Kan. The authors are likewise indebted to these officials for their assistance in collecting and forwarding the monthly water samples.

Clovis, N. Mex.—The common water supply is obtained from three 32-inch, 340-foot wells operated by the New Mexico Utilities Co. All wells are cased their entire depth and apparently obtain water from the "third stratum." There are no "strainers" or perforations in the casings which would permit mixing of water from the first or second stratum. In 1933 the water level stood at about 250 feet below the surface, and prior to that time had been stationary; since then it has fallen 9 feet.

One well was drilled in 1931, one in 1926, and one (city well No. 1) prior to October 1925, when the present operators took over the property from the city. Topographically the wells are located in a triangulated outline, the vertices, marking the location of the wells, being about 200 to 250 feet apart.

Prior to 1925, the municipal water supply was obtained from two "big" wells, 32 inches in diameter and 340 feet deep, known as "city well No. 1" and "city well No. 2," and from four "small" wells, approximately 300 feet deep, one 6 inches in diameter, the other three, 4 inches in diameter. The four "small" wells were abandoned in 1927; "city well No. 2" was abandoned in 1930. Between October 1925 and 1927 about 70 percent of the water supply was obtained from the two 32-inch, 340-foot wells taken over from the city. The remainder came from the four "small" wells.

Webster City, Iowa.—The common water supply is obtained from two deep wells which differ markedly in depth. They are municipally operated. No. 1 is a 10-inch well put in service in 1924 and has been in continuous use since that date. This well is 1,805 feet deep and cased to a depth of 1,520 feet. There are no "slots" or perforations in the casing which would permit mixture of water from higher levels. Water is apparently obtained from three water-bearing sands, 46 feet, 56 feet, and 65 feet in thickness, respectively, located between the 1,605- and 1,790-foot levels. The highest of these aquiferous sands is approximately 150 feet below the St. Peter sandstone. It might be noted that the drilling log indicated that no water was obtained from the St. Peter sandstone in this particular locality.

Well No. 2 is a 12-inch, 120-foot well, likewise drilled in 1924 and in continuous use since that date, with the exception of the period between February 1930 and November 1, 1932. During 1932 this well was deepened from 110 feet to 120 feet. According to the water superintendent, well No. 1 furnishes about two-thirds of the city water, the remainder being supplied by well No. 2.

The water superintendent states that the 12 monthly samples sent to the National Institute of Health were taken from a tap on the high surface pump discharge. This tap is located on a line between the "clear" reservoir and the distribution mains and pressure storage tanks. A water sample from this source is representative of that used by the inhabitants. Samples were generally collected in the forenoon. As a rule, well No. 1 is pumped from midnight to noon and well No. 2 from noon to midnight.

A large number of individual shallow wells are used in the East School district.

East Moline, Ill.—The common water supply of East Moline is obtained from two deep wells, municipally operated. Well No. 1 was installed in 1895, and at that time was 1,300 feet in depth; well No. 2 was drilled in 1911 to a depth of 1,371 feet. In 1913 both wells were deepened, No. 1 to a depth of 1,532 feet, No. 2 to 1,850 feet. There had been no changes in the physical setup between 1913 and the date of the survey, November 1936. Both wells furnish approximately equal amounts to the city supply. Water from the two wells is pumped by air lift into a collecting reservoir, from which it is pumped into the distribution system. Two elevated steel tanks connected with the distribution system have a capacity of 100,000 and 500,000 gallons, respectively.

No local information was obtainable as to the depth of casings or strata from which water is apparently being obtained. During July and August it has been customary to augment the East Moline supply by obtaining come water from the nearby village of Silvis. This supplemental supply amounts to about one-third of the water used by the inhabitants of East Moline during these 2 months. The Silvis supply is obtained from one deep well, 1,985 feet in depth, and from one shallow well, 35 to 40 feet deep. The city clerk of East Moline states, however, that the monthly samples forwarded to the National Institute of Health should not show the possible effects of this change as the East Moline samples were in all cases collected from the East Moline Reservoir. Inasmuch as the distribution systems of the two communities connect at a point some distance from the reservoir, whatever water East Moline might obtain from Silvis should not pass through the East Moline Reservoir.

Junction City, Kans.—The common water supply of Junction City is obtained from three 60-foot wells, installed in August 1924 and in constant use since that date. These wells are concrete cased; the outside casing is 24 inches in diameter, the inside 18 inches. There are strainers in the lower 20 feet of the casing, the upper 40 feet being plain concrete casing. All three wells are located near the Republican River; well No. 1 is about 100 feet from the river, well No. 2 about 200 feet, while well No. 3 is located in the old Republican River Basin, which is now about 125 feet from the present river bank. Each well has a direct-connected deep-well turbine pump and motor. The water is pumped into a collection reservoir from which point the high-service pumps deliver the water to the city distribution system. On the suction side of the high service pumps, chlorine is introduced in varying amounts.

The drilling log indicates that the wells pass through about 25 feet of clay and 15 feet of very fine gravel, while the lower 20 feet is composed of fine to coarse gravel resting on a limestone stratum. The water level in the three wells correlates with the height of water in the nearby Republican River.

METHOD OF CLINICAL SURVEY

Standards of clinical survey, previously described (*1*), were followed. In this particular study the age group consisted of the 9-, 10-, and 11-year-old children, the age grouping being by last birthday. In order to confine the study group exclusively to those children constantly exposed to the risk of the disease, all children who stated that they had not lived in the city continuously since birth (30 days in a calendar year excepted), or had not used the municipal water exclusively, were eliminated. The remainder were then further questioned as to their residence and drinking-water history. This cross-questioning often revealed breaks in continuity of residence, such as living for a time at some nearby town or farm, or vacations away from home in excess of 30 days. There are, furthermore, some instances in which children lived in the community continuously but had obtained their domestic water from other than the public supply. Children whose histories indicated any of these discontinuities were eliminated from further study.

All examinations were made in a good light with the child facing a window. At Webster City, East Moline, and Junction City, mouth mirrors free from blemishes and new explorers (SSW No. 5, or equal) were used in making the examinations. In these three cities, in addition to mottled enamel, other defects of the enamel such as caries, present or past (fillings), pits and fissures hypoplasias, etc., were recorded on an individual record form, reproduced in a previous report (*1*).

At Clovis, the diagnosis of the degree of mottled enamel severity was based on a careful visual examination without the aid of mouth mirrors. The observations were recorded by the "cross five" method on a tally sheet designed for mottled-enamel surveys. The signs of chronic endemic dental fluorosis are so objective that little variation in incidence is noted in using either one or the other of these methods. For instance, in 1935 a test was made of the two methods at Colorado Springs, Colo. In this city there are 10 public grade schools, 5 east of Monument Creek and 5 west of the creek. In the five schools east of Monument Creek there were seventy-nine 9-year-old children who stated that they had resided in the community continuously since birth and had always used the city water supply. An examination of this group using a mouth mirror and explorer disclosed an incidence of 67 percent. An examination of 69 children of the same age and comparable to the first group as to residence and water history in the five schools west of Monument Creek, using the method of careful visual examination without mouth mirrors, showed an incidence of 68 percent. In Clovis the recording of the detailed results of each examination on an individual record form and then verifying the history as given by each child by an interview with the child's parent would have been purposeless in view of the interfering variable in the physical setup of the water

TABLE 2.—*Distribution according to severity of dental fluorosis in those examined in item 4 of table 1*

City	Number of children examined	Children classified according to mottled enamel diagnosis							Incidence per 100 children
		Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe	
		Number							
Clovis, N. Mex.	138	18	22	33	49	15	1	0	71.0
Webster City, Iowa	72	47	6	16	3	0	0	0	26.4
East Moline, Ill.	110	61	22	20	6	1	0	0	24.5
Junction City, Kans.	115	110	3	2	0	0	0	0	1.7
		Percent							
Clovis, N. Mex.	138	13.1	15.9	23.9	35.5	10.9	0.7	0	71.0
Webster City, Iowa	72	65.3	8.3	22.2	4.2	0	0	0	26.4
East Moline, Ill.	110	55.5	20.0	18.2	5.4	0.9	0	0	24.5
Junction City, Kans.	115	95.7	2.6	1.7	0	0	0	0	1.7

TABLE 3.—*Distribution according to severity of dental fluorosis in those remaining after recheck (see item 6 of table 1)*

City	Number of children examined	Children classified according to mottled enamel diagnosis							Incidence per 100 children
		Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe	
		Number							
Clovis, N. Mex.[1]									
Webster City, Iowa	47	26	5	13	3	0	0	0	[2] 34.0
East Moline, Ill.	94	51	19	18	5	1	0	0	25.5
Junction City, Kans.	93	90	1	2	0	0	0	0	2.1
		Percent							
Clovis, N. Mex.									
Webster City, Iowa	47	55.3	10.7	27.7	6.3	0	0	0	34.0
East Moline, Ill.	94	54.3	20.2	19.1	5.3	1.1	0	0	25.5
Junction City, Kans.	93	96.8	1.1	2.1	0	0	0	0	2.1

[1] Clovis data was not rechecked; see text.

[2] The increase in incidence of affection from 26.4 to 34.0 percent as a result of the house-to-house recheck is the largest yet recorded in surveys of this type. The large number of shallow wells in use no doubt contributes to this increase.

TABLE 1.—*Summary of data with relation to continuity of residence and concomitant use of the communal water supply*

	Clovis, N. Mex.	Webster City, Iowa	East Moline, Ill.	Junction City, Kans.
(1) Number of grade public schools in city	2	5	5	3
(2) Number of grade public schools in which examinations were held	2	5	5	3
(3) Number of 9-, 10-, and 11-year old pupils in attendance in (2) on date of examination	424	242	336	[1] 355
(4) Number of pupils in (3) whose histories on questioning indicated constant residence and concomitant use of communal water supply and who were examined	138	72	110	115
(5) Percentage of age group present examined under (4)	32.5	29.7	32.7	32.4
(6) Number of schedules eliminated by house-to-house recheck		25	16	22
(7) Percentage of total present showing constant residence and water history		19.4	27.9	26.2

[1] There were actually 415 children present, but about 20 in each age group (60 in all) were children from Fort Riley, a nearby military reservation; 355 represent approximately the actual number of Junction City residents present.

TABLE 4.—*Fluoride (F) content of monthly samples*

Month and year	Clovis	Webster City	East Moline	Junction City
	Parts per million			
1935				
December	2.1	1.6	1.6	0.7
1936				
January	2.2	1.5	1.6	.7
February	2.3	1.5	1.6	.7
March	2.3	1.6	1.5	.7
April	2.3	1.6	1.5	.7
May	2.3	1.6	1.5	.7
June	2.1	1.6	1.6	.7
July	2.1	1.6	1.6	.7
August	2.2	1.6	1.5	.7
September	2.2	1.5	[1] 1.0	.6
October	2.1	1.5	1.6	.6
November	2.1	1.5	1.6	.6
Mean annual fluoride content	2.19	1.56	[1] 1.51	.67

[1] When the abrupt change in fluoride content of the September sample was noted, inquiry was made as to a possible explanation. Mr. Arnold Neihaus, city clerk of East Moline, furnished the information that 1 of the wells was not used during that time on account of a broken pump. In order to have information as to possible fluctuation in fluoride content on account of the use of only 1 of the wells, samples from each well were sent by Mr. Neihaus for analysis. These results showed that the water from the well used when the September sample was taken had a fluoride (F) content of 1.0 part per million, while the water from the other well showed a fluoride (F) content of 1.6 parts per million. According to local information, each well contributes about equal amounts to the municipal supply. But as indicated by the fluoride figures, the samples sent us apparently were chiefly representative of one or the other of the 2 wells and not an equal mixture of the 2. Hence, the arithmetical mean annual fluoride content might be in the neighborhood of 1.3 parts per million instead of the 1.5 parts per million shown in table 4.

Similar determinations (on single samples) of the fluoride content of the water from each of the 2 wells supplying Webster City, showed that the fluoride (F) content of one was 1.8 parts per million, while that of the other was 1.3 parts per million.

supply. There is no object in expending time and effort in obtaining the data upon which an "actual" mottled enamel index is computed when major changes in the water supply contravene subsequent correlation with the chemical findings. Whether or not the four "small" wells abandoned in 1927 and "city well No. 2," abandoned in 1930, contained a greater or lesser amount of fluoride than the present supply, is, of course, at this time conjectural. The computation of an "actual" mottled-enamel index of a community is justified only in such communities as possess the requisites for quantitative evaluation (*2*).

Following the examinations at Webster City, East Moline, and Junction City, the home of each child was visited and the information recorded on the schedule (*1*) under "Water history" was carefully rechecked by an interview with the child's parent for the purpose of later computing an "actual" mottled-enamel index of the community. This recheck often revealed additional inaccuracies in residence or water supplies which the child either did not know or had forgotten. Under the conditions of this survey in the three cities above mentioned, it was possible to find only 19.4, 27.9, and 26.2 percent, respectively, of the children who could demonstrate a continuous residence and an exclusive use of the city water during their lifetime. A previous survey (*1*) of four other cities, but limited to the 9-year-old group, showed 20.4, 20.8, 21.3, and 22.8 percent with verified continuous residence and constant use of the communal water supply. The striking fact that such a high percentage of school children shows discontinuities in residence or noncontinuous use of a common water supply cannot be overstressed in mottled-enamel surveys made for the purpose of determining the incidence associated with a particular fluoride concentration or a subsequent computation of a mottled-enamel index. A survey of a group of school children, without adjusting for this time-of-exposure factor, naturally results in a rate of affection considerably less than the actual rate.

The results obtained are summarized in tables 1, 2, and 3.

RESULTS OF CHEMICAL ANALYSIS

As has been noted previously, samples of the waters were obtained monthly. The fluoride content was estimated colorimetrically by means of the zirconium-alizarin reagent (*4*). The results obtained are given in table 4.

As was customary in other quantitative surveys, analyses were made of constituents of the water other than the fluorides. Results of the chemical analyses of the waters are given in table 5.

Check analyses on the mineral content, comprising determinations of total solids, alkalinity, chloride, and sulphate, were made on each of the monthly samples. The results indicated that the gross mineral composition was practically uniform in all of the monthly water samples from each community, with the exception of the September sample from East Moline. (See footnote to table 4).

DISCUSSION

There are numerous references in the literature to surveys of mottled enamel areas in which little data is submitted relative to the size of the sample, the age distribution, or the continuity of exposure of the group upon which inferences are postulated. In surveys of communities with populations of 1,500 or less, the adequacy of the sample becomes one of paramount importance. Even though all 9-, 10-, 11-, and 12-year-old children of continuous residence and a history of constant use of the common

TABLE 5.—*Analyses of the waters*[1] *used*

	Clovis	Webster City	East Moline	Junction City
	Parts per million			
Residue on evaporation	265.8	729.0	1,128.6	400.5
Loss on ignition	23.3	126.8	46.5	36.2
Fixed residue	242.5	602.2	1,082.1	364.3
Silica (SiO_2)	35.9	14.5	12.2	27.7
Iron (Fe)	.02	.18	.08	.04
Aluminum (Al)	.2	.4	.1	.3
Calcium (Ca)	25.7	105.5	54.3	83.5
Magnesium (Mg)	21.8	50.5	25.7	17.9
Sodium and potassium (calculated as Na)	40.2	60.2	309.3	29.7
Bicarbonate (HCO_3)	234.9	406.9	308.0	294.6
Sulphate (SO_4)	24.2	235.6	290.9	45.9
Nitrate (NO_3)	7.9	3.5	3.0	4.8
Chloride (Cl)	16.5	25.0	259.0	31.0
Fluoride (F)	2.2	1.5	1.4	.7
Phosphate (PO_4)	0	.2	0	.2
Boron (B)	0	0	0	0

[1] The sample of water from Clovis was collected in September 1935, the East Moline and Webster City samples in October 1935, and the Junction City sample in November 1935.

Assistant Chemist C. G. Remsburg carried out the determinations other than fluoride and boron, using mostly the methods given in the Standard Methods of Water Analysis of the American Public Health Association. The boron determinations were made essentially by the method of Foote (*5*).

water supply are examined, the group is generally so small that extreme caution is warranted in interpreting the results. Comments relative to a few of these observations follow.

Table 7.—*Relation between the fluoride (F) concentration and clinical effect in 9-year-old children with verified histories in 6 selected cities*

City and State	Number examined	Mean annual fluoride (F) content of common water supply (p. p. m.)	Incidence per 100 children
Colorado Springs, Colo.	54	2.5	66.6
Monmouth, Ill.	[1] 29	1.7	48.3
Webster City, Iowa	[1] 20	1.6	25.0
East Moline, Ill.	[1] 35	[2] 1.5	17.1
Junction City, Kans.	[1] 30	.7	3.3
Pueblo, Colo.	46	.6	4.0

[1] The number examined in these 4 cities was less than our minimal requisite of 50 when the incidence is less than 75 percent, but represents all of the children of this specific age group present in the public schools on the day of the examination who had a verified history of constant residence and continuous use of the municipal water. For importance of size of samples, see table 8.

[2] See footnote, table 4.

Effect on Incidence of Age Grouping of Sample

Analyses of data from numerous surveys indicate that when dealing with fluoride (F) concentrations between 1.0 and approximately 2.5 parts per million, an examination of the 9-year-old children exclusively shows a lower incidence than when the examination includes the 9-, 10-, and 11-, or the 9-, 10-, 11-, and 12-year-old children. A possible explanation of this phenomenon has previously been suggested (*3*):

> Two related factors are probably the cause of this somewhat lower incidence in a survey limited to the 9-year-age group. First, in endemic areas of relatively low fluoride concentration (less than 2 parts per million) there is, in a fair proportion of the children of comparable and constant residence and water history, a tendency to show the milder forms of mottled enamel only on the bicuspids and second molars, a group of teeth which, according to Kronfeld (Development and Calcification of the Human Deciduous and Permanent Dentition. The Bur, March 1935) began their calcification at a somewhat later date than the incisor-first molar group. This manifestation of mild dental fluorosis in teeth calcified at a somewhat later date is suggestive of a cumulative action of fluorine. Second, based on an analysis of the 162 schedules of the Colorado Springs-Pueblo survey (*1*) only about 1 percent of the permanent second molars, 7.2 percent of the second bicuspids, and 20.5 percent of the first bicuspids were erupted in the 9-year-age group. It follows, therefore, that certain 9-year-old children are necessarily classified as normal on the basis of the absence of mottled enamel on the incisor-first molar group when, if the same individual were examined a year or two later, it might show objective signs of mottled enamel on the bicuspid-second molar group and be so classified.

Illustrations of this tendency may be seen in table 6.

Comparison of Present Study with a Previous One

Keeping in mind the probabilities of a somewhat lower percentage incidence of affection in certain samples limited to 9-year-old children (see table 6), it seems desirable to compare the findings of this study with one previously reported (*1*). As the earlier work (Colorado Springs-Monmouth-Pueblo) was limited to the 9-year-age group, the comparison will be made only with the same age group in Webster City, East Moline, and Junction City. In the 213 observations listed, each child was born in the community, lived there all of his or her life, used the communal water supply continuously for both drinking and cooking; and the facts in each instance were verified by one of us (HTD) by an interview with the child's parent.

Table 6.—*Difference in the percentage incidence between samples composed of 9-year-old children exclusively and samples consisting of 9-, 10-, and 11-year-old children*

City and State	Mean annual fluoride content (p. p. m.)	9-year-old children		Composite of 9-, 10-, 11-year-old children		Difference in incidence
		Number examined	Incidence per 100 children	Number examined	Incidence per 100 children	
Pueblo, Colo.	0.6	83	2.4			
Junction City, Kans.	.7	38	2.6	115	1.7	−0.9
East Moline, Ill.	1.5	42	19.0	110	24.5	+5.5
Webster City, Iowa	1.6	33	15.1	72	26.4	+11.3
Monmouth, Ill.	1.7	38	42.1			
Clovis, N. Mex.	2.2	45	57.7	138	71.0	+13.3
Colorado Springs, Colo.	2.5	79	67.1			
Plainview, Tex.	2.9	30	86.6	77	87.0	+.4
Amarillo, Tex.	3.9	77	87.0	229	89.5	+2.5
Conway, S. C.	4.0	23	86.9	59	88.1	+1.2
Lubbock, Tex.	4.4	70	95.7	164	97.6	+1.9

Note.—In the observations listed in table 6, the histories elicited from each child by careful cross-questioning indicated constant residence and continuous use of the common water supply. This is the standard, followed in recording the data used in determining the "approximate mottled enamel index" (*6*). The variation in the incidence between this method and that of verifying each history by an interview with the child's parent (actual mottled enamel index) is generally of minor importance. In the cases of Junction City, East Moline, and Webster City, the differences may be noted in tables 2 and 3. The variation with respect to Pueblo, Monmouth, and Colorado Springs has been reported in a previous publication (*1*). In Conway the house-to-house recheck had a negligible effect on the group incidence, the 59 cases shown in table 6 revealing an incidence of 88.1, while the 26 verified rechecked schedules showed an incidence of 88.4 per 100 children.

Sampling Limits

In evaluating the degree of reliability attached to percentage incidences computed on groups not meeting the present minimal requirements as to size, it is well to bear in mind the probability of fluctuation in small samples. Extensive data distributed over an adequate number of surveys have not as yet been accumulated in amounts sufficient to warrant the development of sampling limits in relation to waters of different fluoride concentrations. The magnitude and composition of the sample naturally bears a direct relationship to the percentage of affection of the particular endemic area being studied.

Pending the collection of further data we follow this standard with respect to size and composition of the sample used in the computation of an "actual" or an "approximate" community mottled enamel index. This index is merely a numerical ratio (*7*) of measurement that points out the approximate percentage distribution of clinical severity observed in the group at the time of the examination. The group examined is limited to children who since birth have continuously used the common water

TABLE 8.—*Examples of fluctuation in percentage incidence in samples of different sizes and combinations in (a) the same endemic area, and (b) endemic areas showing different fluoride concentrations*

Age group	Number of children examined	Designation of school	Classification of mottled enamel diagnosis: Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe	Incidence of mottled enamel per 100 children
Lubbock, Tex. Mean annual fluoride (F) content, 4.4 p. p. m.										
9-year-old children	20	School A	0	1	4	6	7	2	0	95
	18	School B	1	0	2	3	8	4	0	94
	19	School C	0	1	1	4	10	2	1	95
	13	Schools D[1]	0	0	1	5	6	1	0	100
	70	All schools	1	2	8	18	31	9	1	95.7

Percentage incidence in different combinations

Schools AB (38)	95	Schools CD (32)	97
Schools AC (39)	95	Schools ABC (57)	95
Schools AD (33)	97	Schools ABD (51)	96
Schools BC (37)	95	Schools BCD (50)	96
Schools BD (31)	97	Schools CDA (52)	96

Age group	Number of children examined	Designation of school	Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe	Incidence of mottled enamel per 100 children
Amarillo, Tex. Mean annual fluoride (F) content, 3.9 p. p. m.										
9-year-old children	19	School A	1	3	3	5	4	3	0	79
	17	School B	0	0	2	8	7	0	0	100
	25	School C	1	3	3	7	9	2	0	84
	16	School D	2	0	2	3	6	2	1	87
	77	Schools A, B, C, D	4	6	10	23	26	7	1	87

Percentage incidence in different combinations

Schools AB (36)	89	Schools CD (41)	85
Schools AC (44)	82	Schools ABC (61)	87
Schools AD (35)	83	Schools ABD (52)	88
Schools BC (42)	90	Schools BCD (58)	90
Schools BD (33)	94	Schools CDA (60)	83

[1] Schools "D" — a combination of scattering cases from 3 schools.

supply for both drinking and cooking, breaks of continuity totaling less than 30 days in any one calendar year being excepted.

Actual mottled enamel index.—The sample of children upon whom the index is based must consist of 25 or more, 9 years of age or older. Whenever possible, the sample should consist of 9-, 10-, 11-, and 12-year-old children, represented in approximately equal numbers. In instances where the examination of the first 25 reveals an incidence of less than 75 percent, the size of the sample must be increased to 50 or more to compensate for fluctuations in sampling. All histories as given by the child with respect to both residence and water supplies must be rechecked and confirmed by an interview with the child's parent. This index is computed only when there have been no relevant changes in the physical setup of the common water supply concomitant with the life period of the group examined.

Approximate mottled enamel index.—The minimal requisites with respect to numbers examined and age distribution as given for an "actual mottled enamel index" are followed, but individual histories are not rechecked by an interview with the child's parent. The approximate mottled enamel index is particularly useful in routine surveys. In surveys of small communities we have frequently computed what is known as a "tentative mottled enamel index" as the number of children with continuity of exposure is insufficient to compute an "actual" or "approximate" index. The sample in this instance consists of not less than 10 but not more than 24 children. Numerous surveys have shown that after careful cross-questioning of each individual child, the remaining error with respect to either residence or water history is largely compensatory and the house-to-house recheck as required for the "actual mottled enamel index," results in little change in either the percentage of affection or the percentage distribution of severity of the group as a whole.

Instances of fluctuation in incidence in samples of different sizes and combinations in the case of four cities with mean annual fluoride (F) contents of 4.4, 3.9, 2.5, and 1.5 parts per million, respectively, are shown in table 8.

APPLICABILITY OF THE SEVEN-GRADE CLASSIFICATION OF DIAGNOSIS

In 1934 there was published a classification of mottled enamel diagnosis (*8*). At that time the classification was based upon approximately 2,000 observations made in endemic areas of six different States. The primary purpose in developing this classification was to provide a standard of measurement for recording the degree of severity as observed in communities whose water supplies contained various concentrations of fluorides. To date, its applicability has been tested by one of us (HTD) in more than 10,000 examinations in about 185 areas distributed among 16 States. A large majority of these were endemic areas; but in connection with these surveys the classification has of course, been applied in the study of borderline and negative, or "control," areas.

Certain objections have been raised to the use of this classification on the grounds that seven grades tend to make the classification complex. Some have suggested a classification of "mild," "moderate," and "severe" in the interest of simplification. This proposal is evidently based on the erroneous assumption that the entire classification is to be used in a survey of any particular endemic area. Such is not the case.

In considering the simplified classification, "mild," "moderate," and "severe," we would naturally have to add a fourth grade, "normal" in order to obtain the figure upon which the percentage incidence might be computed, or to show that the recorded frequency of occurrence of mottled enamel plus the frequency of no mottled enamel equals the total number of observations. In other words, it may be taken for granted that the classification could not be reduced to less than four grades under any conditions.

Those who would simplify the classification would omit the "questionable," "very mild," and "moderately severe" grades. The quantitative aspects of a survey would probably be entirely lost, or judged erroneously, if the "questionable" cases were thrown into one grade or another of the proposed four-grade classification. Similarly, the omission of the "very mild" and "moderately severe" grades would probably seriously affect the calculation of the community mottled enamel index. This retrogression from quantitative methods would result in abstruse survey data of little value for purposes of comparison with other endemic areas of greater or lesser degree of severity.

An analysis of the data from 15 surveys, totaling 1,542 observations, indicates the flexibility of the seven-grade classification. In these 15 cities, where the percentage incidence ranges from 2.4 to 100, it is noted that more than 90 percent of all diagnoses fall into not more than four grades (arithmetical mean, 95.3; weighted average mean 92.0). A cursory analysis of table 9 makes apparent the flexible features of a seven-grade classification and its applicability to areas of dissimilar degrees of clinical severity.

CONCLUSION

In accordance with previously described means (7) of determining the mottled enamel index of a community, the application of this method to the percentage distribution of severity as listed in table 2 indicates that the approximate mottled enamel index of Clovis, N. Mex., is "slight," that of Webster City, Iowa, and East Moline, Ill., "border line," and that of Junction City, Kans., "negative." The application of these same ratios to the rechecked verified data in table 3 shows that the actual mottled enamel index of East Moline, Ill. is "border line" and that of Junction City, Kans., "negative." The percentage distribution of severity, however, in the case of Webster City, Iowa, shows an approach so close to the next higher index that the actual mottled enamel index of this city should be listed as "border line—slight."

Table 8.—*Examples of fluctuation in percentage incidence in samples of different sizes and combinations in (a) the same endemic area, and (b) endemic areas showing different fluoride concentrations*—Continued

Age group	Number of children examined	Designation of school	Classification of mottled enamel diagnosis							Incidence of mottled enamel per 100 children
			Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe	
Colorado Springs, Colo. Mean annual fluoride (F) content, 2.5 p. p. m.										
9-year-old children..	13	School A........	2	3	3	2	3	0	0	62
	23	School B........	7	3	6	4	2	1	0	56
	15	School C........	4	2	6	1	1	1	0	60
	15	School D........	1	2	4	5	3	0	0	80
	13	School E........	1	1	5	2	4	0	0	85
	79	Schools, A, B, C, D, E.........	15	11	24	14	13	2	0	67.1
		Percentage incidence in different combinations								
		Schools AB (36)....	58			Schools ABC (51)....	59			
		Schools AC (28)....	61			Schools ABD (51)....	65			
		Schools AD (28)....	71			Schools ABE (49)....	65			
		Schools AE (26)....	73			Schools BCD (53)....	64			
		Schools BC (38)....	58			Schools BCE (51)....	65			
		Schools BD (38)....	66			Schools CDE (43)....	74			
		Schools BE (36)....	67			Schools CDA (43)....	67			
		Schools CD (30)....	70			Schools DEA (41)....	76			
		Schools CE (28)....	71			Schools DEB (51)....	71			
		Schools DE (28)....	82			Schools EAC (41)....	68			
East Moline, Ill. Mean annual fluoride (F) content, 1.5 p. p. m.[3]										
9-, 10-, and 11-year-old children.	24	School A........	14	5	3	2	0	0	0	21
	40	School B........	22	8	9	1	0	0	0	25
	19	School C........	11	1	6	1	0	0	0	37
	10	School D........	5	2	1	1	1	0	0	30
	17	School E........	9	6	1	1	0	0	0	12
	110	All schools.	61	22	20	6	1	0	0	24.5
		Percentage incidence in different combinations								
		Schools AB (64)....	23			Schools ABC (83)....	26			
		Schools AC (43)....	28			Schools ABD (74)....	24			
		Schools AD (34)....	23			Schools ABE (81)....	21			
		Schools AE (41)....	17			Schools BCD (69)....	29			
		Schools BC (59)....	29			Schools BCE (76)....	25			
		Schools BD (50)....	26			Schools CDE (46)....	26			
		Schools BE (57)....	21			Schools CDA (53)....	28			
		Schools CD (29)....	34			Schools DEA (51)....	20			
		Schools CE (36)....	25			Schools DEB (67)....	22			
		Schools DE (27)....	19			Schools EAC (60)....	23			

[3] See footnote to table 4.

Note.—Number in parenthesis following each combination indicates the size of the sample.

Analysis of the data in this study confirms the previous statement (*3*) that "amounts not exceeding 1 part per million expressed in terms of fluorine (F) are of no public health significance."

SUMMARY

1. The "approximate" mottled enamel index of Clovis, N. Mex., is "slight"; that of Webster City, Iowa, and East Moline, Ill., "border line"; while that of Junction City, Kans., is "negative." The "actual" mottled enamel indexes of East Moline and of Junction City are the same as the approximate indexes; but the "actual" index of Webster City approaches so close to the next higher classification that it is listed as "border line—slight."

2. The mean annual fluoride (F) content based upon monthly examinations of the common water supply received between December 1935, and November 1936, was for Clovis, N. Mex., close to 2.2 parts per million; for Webster City, Iowa, 1.6 parts per million; for East Moline, Ill., 1.5 parts per million (subject to certain corrections which might bring it down to about 1.3 parts per million); and for Junction City, Kans., 0.7 parts per million.

3. Problems of sampling, particularly with respect to adequateness, are discussed and illustrated with examples from surveys of cities whose common water supplies show different fluoride concentrations.

4. The need, for quantitative purposes, of the "seven-grade" classification of mottled enamel diagnosis is shown.

5. Additional evidence is presented that amounts of fluoride (F) not exceeding 1 part per million are of no public health significance.

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TABLE 9.—*Illustration of (a) the tendency of mottled enamel diagnoses to fall into 4 grades (as indicated by figures in boldface type), and (b) the adaptability of the 7-grade classification to meet the survey needs in communities having waters of different fluoride concentrations*

City and State	Number of children examined	Mean annual fluoride (F) content in p. p. m.	Percentage incidence of affection	Percentage distribution of clinical severity							Highest percentage of diagnoses falling into not over 4 grades	Age group or school grade examined
				Pathognomonic signs								
				Absent		Present						
				Normal	Questionable	Very mild	Mild	Moderate	Moderately severe	Severe		
Pueblo, Colo	83	0.6	2.4	**91.6**	**6.0**	**2.4**					100.0	9-year old.
Junction City, Kans	115	.7	1.7	**95.7**	**2.6**	**1.7**					100.0	9-, 10-, 11-year old.
Mullins, S. C	47	.9	10.6	**68.1**	**21.3**	**8.5**	2.1				100.0	Do.
East Moline, Ill	110	[1] 1.5	24.5	**55.5**	**20.0**	**17.3**	**6.4**	0.9			99.1	Do.
Webster City, Iowa	72	1.6	26.4	**65.3**	**8.3**	**22.2**	**4.2**				100.0	Do.
Monmouth, Ill	38	1.7	42.1	**36.8**	**21.0**	**36.8**	**5.3**				100.0	9-year old.
Galesburg, Ill	57	1.8	35.1	**45.6**	**19.3**	**26.3**	**5.3**	3.5			96.5	Do.
Clovis, N. Mex	179	2.2	72.1	11.7	**16.2**	**21.8**	**36.9**	**12.3**	1.1		87.2	9-, 10-, 11-, 12-year old.
Colorado Springs, Colo	148	2.5	67.6	**18.2**	**14.2**	**28.4**	**21.6**	**14.2**	3.4		[2] 82.4	9-year old.
Plainview, Tex	97	2.9	87.6	4.1	**8.3**	**34.0**	**27.8**	**22.7**	3.1		92.8	9-, 10-, 11-, 12-year old.
Amarillo, Tex	289	3.9	90.3	3.1	6.6	**15.2**	**28.0**	**33.9**	**11.1**	2.1	88.2	Do.
Conway, S. C	59	4.0	88.2	5.1	6.7	**30.5**	**22.0**	**23.7**	**11.9**		88.2	9-, 10-, 11-year old.
Lubbock, Tex	189	4.4	97.8	1.1	1.1	**12.2**	**21.7**	**46.0**	**15.3**	2.6	95.2	9-, 10-, 11-, 12-year old.
Post, Tex	38	5.7	100.0				**10.5**	**50.0**	**34.2**	**5.3**	100.0	4th, 5th, 6th grades.
Ankeny, Iowa	21	[3] 8.0	100.0				**9.5**	**47.6**	**33.3**	**9.5**	100.0	Second to twelfth grades, inclusive.

[1] See footnote to table 4.

[2] The percentage distribution of severity in decreasing order is equal in the fourth and fifth tabular cell, 14.2.

[3] Based upon a single chemical determination; all others represent arithmetical mean annual fluoride (F) content.

MOTTLED ENAMEL SURVEY OF BAUXITE, ARK., 10 YEARS AFTER A CHANGE IN THE COMMON WATER SUPPLY

By H. TRENDLEY DEAN, FREDERICK S. MCKAY, and ELIAS ELVOVE

Bauxite, Ark., occupies a prominent place in mottled enamel history. It is a small mining community that was established in 1901 to provide homes and a social environment for the employees of the mining company. It is owned by the Republic Mining and Manufacturing Company, a subsidiary of the Aluminum Company of America and is located about 25 miles southwest of Little Rock. The present population is estimated to be about 1,800.

Apparently the first to report endemic mottled enamel at Bauxite was Dr. F. L. Robertson, a practicing dentist of Benton, a city about 5 miles from Bauxite. His report was made to the Arkansas State Board of Health, and in March 1926 the State health officer asked the United States Public Health Service to make a study of mottled enamel in Bauxite.

The original domestic water supply of Bauxite was obtained from shallow wells and a few springs. With the increase in population and contamination of certain of these surface supplies, a new water supply became necessary, and in 1909 two 297-foot wells were drilled. One of these wells became partly filled at the bottom from caving, and in September 1925 a new 245-foot well was added.

It appears that the association of mottled enamel with the use of water from the deep wells was recognized even before a survey of the community had been made; Mr. L. R. Branting, superintendent, wrote to one of us (F. S. McK.) as early as September 1927 that steps had already been taken to change the common water supply. He states: "In choosing our new supply of water, we took account of the fact that the city of Benton gets its domestic water from the Saline River and that this water does not seem to affect the teeth of the children. At the time of the 1928 survey, the public school children at Benton were also examined and the fact established that mottled enamel was not associated with the use of the Saline River water. These observations were included in the report of the original survey at Bauxite. He further added that a 6-inch pipe leading from the Saline River, 7 miles distant, had already been installed and that construction had been started on a filter to render the water suitable for domestic purposes.

In February 1928 a survey of mottled enamel was made by Kempf and McKay (*1*). In this survey 458 children, ages 5 to 18, were examined in the schools of Bauxite. Mottled enamel of some teeth was found in 202 cases, or 44 percent. Among the 458 children examined, there were 66 children, ages 5 to 18 years, who, the authors state, were born in Bauxite, had lived there all of their lives, and had always used the municipal deep-well supply. Of these 66 children, 63 had some permanent teeth erupted. Sixty-one of the 63 showed evidence of mottled enamel. It was also noted in this report that the homes of each of the two

TABLE 1.—*Summary of data with relation to continuity of residence and concomitant use of the Bauxite common water supply*

	Grades				Total
	1-2	3-4	5-6	7-8	
1. Number of pupils in attendance on the day of screening	73	75	71	89	308
2. Number of pupils whose history on questioning indicated constant residence and continuous use of the communal water supply and who were examined	32	30	20	24	106
3. Percentage of pupils present examined under (2)	44	40	28	26	34
4. Number of schedules eliminated by house-to-house check	7	8	4	5	[1] 24
5. Percentage of total present showing verified constant residence and water history	34	29	22	21	26

[1] The most frequent single cause for rejection was the continued use of water from the "Hamp Smith Spring" for drinking prior to 1928. Eight of the 24 schedules eliminated were for this reason.

children with normal teeth were piped for the common water supply, but one gave a history of not using this water for either cooking or drinking, while the other is recorded as having used spring water for drinking most of the time.

The evidence collected by these investigators in the examination of the elementary and high school children was summarized as follows:

"1. No cases of the enamel defect were found which antedated the introduction of the deep-well water.

"2. The oldest individual found with this enamel defect was born about the time that the deep-well water was introduced.

"3. All individuals in the community who had used the deep-well water during any considerable period of enamel formation exhibited this defect.

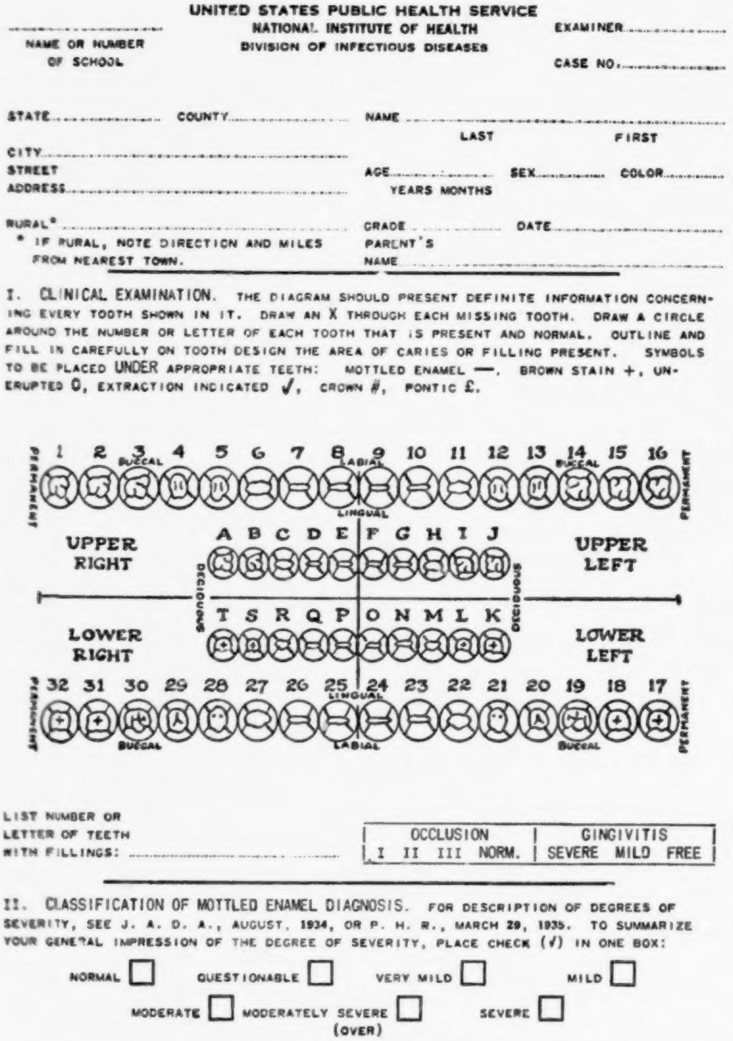
"4. No individual in the community whose enamel had developed elsewhere exhibited this defect.

"5. Certain individuals, who, although residents of the community and attending school there, but who actually lived beyond the distribution of the deep-well water and depended upon the original shallow wells, exhibited only normal enamel."

In 1931 Churchill (*2*) stated that, following the publication of the report of Kempf and McKay (Nov. 28, 1930), "A. W. Petrey of this laboratory (Aluminum Research Laboratories, New Kensington, Pa.) spectrographically discovered the presence of fluorides in deep-well water from Bauxite, Ark." In the same article Churchill reported that a quantitative determination of the fluorine content of the Bauxite deep-well water showed 13.7 parts per million.

The use of filtered river water began in May 1928, and has been continuous since that time. Of the two deep wells from which water was obtained prior to the time of the change, one, the 245-foot well, is still used for industrial purposes, the other having been abandoned. Water from the deep well may be pumped into the distribution system, but it would necessitate the repair of a relift pump and manipulation of a one-gate valve. There is no record of this having been done since the installation of the filtered river water in 1928. Practically all of the population use the common water supply.

Adjoining Bauxite is a small mining community known as Norton Town. The water supply for this community is obtained from a well 247 feet deep located about 300 to 400 yards from the old Bauxite deep wells and apparently obtaining water from the same stratum. The water from this supply is piped to the Norton Town homes. At the time of the resurvey (Mar. 1938), there were 14 families using this water. The Norton


UNITED STATES PUBLIC HEALTH SERVICE
NATIONAL INSTITUTE OF HEALTH
DIVISION OF INFECTIOUS DISEASES

NAME OR NUMBER OF SCHOOL ....
EXAMINER ....
CASE NO. ....

STATE .... COUNTY ....
NAME .... LAST FIRST
CITY ....
STREET ADDRESS ....
AGE .... YEARS MONTHS
SEX ....
COLOR ....
RURAL* ....
* IF RURAL, NOTE DIRECTION AND MILES FROM NEAREST TOWN.
GRADE ....
DATE ....
PARENT'S NAME ....

I. CLINICAL EXAMINATION. THE DIAGRAM SHOULD PRESENT DEFINITE INFORMATION CONCERNING EVERY TOOTH SHOWN IN IT. DRAW AN X THROUGH EACH MISSING TOOTH. DRAW A CIRCLE AROUND THE NUMBER OR LETTER OF EACH TOOTH THAT IS PRESENT AND NORMAL. OUTLINE AND FILL IN CAREFULLY ON TOOTH DESIGN THE AREA OF CARIES OR FILLING PRESENT. SYMBOLS TO BE PLACED UNDER APPROPRIATE TEETH: MOTTLED ENAMEL —, BROWN STAIN +, UNERUPTED O, EXTRACTION INDICATED ✓, CROWN #, PONTIC £.

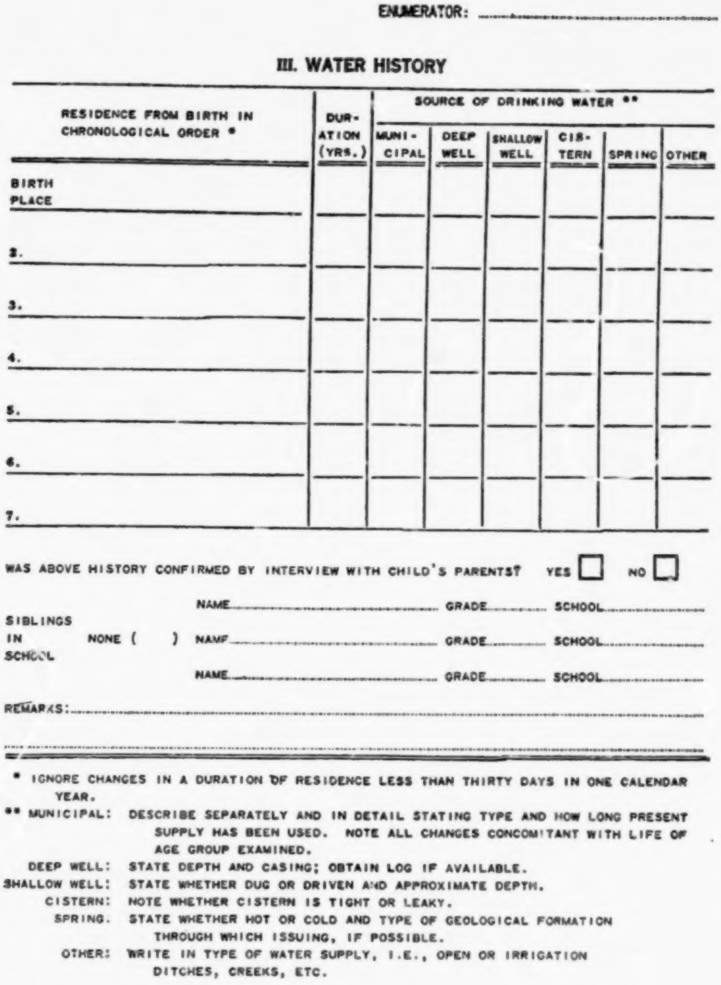
LIST NUMBER OR LETTER OF TEETH WITH FILLINGS: ....

OCCLUSION	GINGIVITIS
I II III NORM.	SEVERE MILD FREE

II. CLASSIFICATION OF MOTTLED ENAMEL DIAGNOSIS. FOR DESCRIPTION OF DEGREES OF SEVERITY, SEE J. A. D. A., AUGUST, 1934, OR P. H. R., MARCH 29, 1935. TO SUMMARIZE YOUR GENERAL IMPRESSION OF THE DEGREE OF SEVERITY, PLACE CHECK (✓) IN ONE BOX:

NORMAL ☐ QUESTIONABLE ☐ VERY MILD ☐ MILD ☐
MODERATE ☐ MODERATELY SEVERE ☐ SEVERE ☐
(OVER)

FIGURE 1a (Obverse).



ENUMERATOR: ....

III. WATER HISTORY

RESIDENCE FROM BIRTH IN CHRONOLOGICAL ORDER *	DURATION (YRS.)	SOURCE OF DRINKING WATER **					
		MUNICIPAL	DEEP WELL	SHALLOW WELL	CISTERN	SPRING	OTHER
BIRTH PLACE							
2.							
3.							
4.							
5.							
6.							
7.							

WAS ABOVE HISTORY CONFIRMED BY INTERVIEW WITH CHILD'S PARENTS? YES ☐ NO ☐

SIBLINGS IN SCHOOL NONE ( )
NAME.............................. GRADE.......... SCHOOL..............
NAME.............................. GRADE.......... SCHOOL..............
NAME.............................. GRADE.......... SCHOOL..............

REMARKS:..

* IGNORE CHANGES IN A DURATION OF RESIDENCE LESS THAN THIRTY DAYS IN ONE CALENDAR YEAR.

** MUNICIPAL: DESCRIBE SEPARATELY AND IN DETAIL STATING TYPE AND HOW LONG PRESENT SUPPLY HAS BEEN USED. NOTE ALL CHANGES CONCOMITANT WITH LIFE OF AGE GROUP EXAMINED.
DEEP WELL: STATE DEPTH AND CASING; OBTAIN LOG IF AVAILABLE.
SHALLOW WELL: STATE WHETHER DUG OR DRIVEN AND APPROXIMATE DEPTH.
CISTERN: NOTE WHETHER CISTERN IS TIGHT OR LEAKY.
SPRING. STATE WHETHER HOT OR COLD AND TYPE OF GEOLOGICAL FORMATION THROUGH WHICH ISSUING, IF POSSIBLE.
OTHER: WRITE IN TYPE OF WATER SUPPLY, I.E., OPEN OR IRRIGATION DITCHES, CREEKS, ETC.

Town families use the Saline River water, the present Bauxite water supply, for drinking, and Norton Town deep-well water for cooking. There is no known connection between these two supplies. Children from Norton Town using this dual domestic water supply were excluded from this study.

The present Bauxite common water supply is obtained from the Saline River. It is pumped from the river to Bauxite, subjected to rapid sand filtration, and disinfected with liquid chlorine. This supply is used almost wholly for domestic purposes, although there are some fire hydrants connected with the treated water system in the residential districts. The major fire protection for the manufacturing plants, however, is carried from the main raw water line from the river. As has been noted, water for industrial purposes is also being obtained from one of the old deep wells. There is, however, no record of a connection between any of these water systems.

RESURVEY OF BAUXITE

Approximately 10 years after the change in the communal water supply, Bauxite was resurveyed. A brief description will be given of the criteria followed in the selection of the sample and the standards used in the clinical survey. The study included all children in the Bauxite school system, first to eighth grades, inclusive, whose history indicated continuous use of the communal water supply. The age distribution of the group was sufficiently wide to permit its subsequent division into three categories, namely, (*a*) children who, during the period of tooth calcification had used the filtered river water exclusively; (*b*) those who had used both the river water and the old deep-well water; and (*c*) those who had used the old deep-well supply almost exclusively.

Each of the class rooms was visited and the purpose of the study carefully explained. Children who stated that they were born in Bauxite, had continuously resided there since birth (30 days in any calendar year excepted), and who had always used the common water supply were assembled in a separate group. This group was then subjected to further interrogation in an attempt to learn of discontinuities in residence or in the use of the common water supply. Judicious questioning often elicited information such as the fact that some children had lived for a time at some nearby town or farm or had used domestic water from a source other than the public supply. Children whose histories indicated any of these discontinuities were eliminated from further study by this screening method. Those remaining apparently represented a group constantly exposed to the risk of water-borne endemic hypoplasia of enamel under investigation, and were accordingly listed for detailed dental examination.

The clinical examinations were made in a good light with the child seated facing a window. Mouth mirrors, free from blemishes, and explorers (Nos. 5, 11, and 12) were used in making examinations. Each child was examined independently by each of the field observes (H.T.D. and F.S.McK.), two schedule forms being prepared for each child. In order to include in our series the largest number possible of children of verified continuity of exposure, 10 children consisting mostly of those absent on the days that the clinical examinations were being made, were examined a day or two later and added to the series of 72 children of continuous history examined independently by each examiner. The findings were recorded by an amanuensis on the special examination form designed for mottled enamel surveys (fig. 1). In addition to mottled enamel, other defects of the enamel such as caries, present or past (fillings), pits and fissures, and hypoplasias of other types were recorded. The dental examinations were made at the approximate rate of 40 per school day per examiner.

Following the clinical examination, the home of each child was visited and the information recorded on the form shown in figure 1 under the heading "III Water History" was carefully rechecked by an interview with the child's parent. This recheck revealed additional inaccuracies in residence or water supplies used, which the child either did not know or had forgotten. Under the conditions of this study, it was possible to find only 82 children continuously exposed to the common water supply of Bauxite with the facts confirmed by the child's parent.

FIGURE 2
A SUMMARY OF TWO SURVEYS OF MOTTLED ENAMEL
MADE IN BAUXITE, ARKANSAS IN 1928 AND IN 1938.
(TEN YEARS AFTER A CHANGE IN THE COMMON WATER SUPPLY)

ITEM		YEAR OF BIRTH OF CHILDREN EXAMINED																									
		YEARS DURING WHICH DEEP WELL WATER WAS USED															YEARS DURING WHICH FILTERED RIVER WATER WAS USED										
		FIRST GROUP OF TEETH (1st MOLARS, INCISORS, AND 1st BICUSPIDS) CALCIFIED WHILE USING WELL WATER.												TRANSITIONAL CALCIFICATION			FIRST GROUP OF TEETH (1st MOLARS, INCISORS, AND 1st BICUSPIDS) CALCIFIED WHILE USING FILTERED RIVER WATER										
		1913	1914	1915	1916	1917	1918	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938
AGE IN YEARS OF CHILDREN EXAMINED AT TIME OF FEB. 1928 SURVEY		15	14	13	12	11	10	9	8	7	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AGE IN YEARS OF CHILDREN EXAMINED AT TIME OF MAR. 1938 SURVEY		-	-	-	-	-	-	-	-	-	-	15	14	13	12	11	10	9	8	7	6	-	-	-	-	-	-
FEB. 1928 SURVEY	NUMBER EXAMINED	1	3	7	2	2	4	8	10	13	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PERCENT POSITIVE ↓	100	100	100	100	100	100	100	100	100	89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MAR. 1938 SURVEY	NUMBER EXAMINED	-	-	-	-	-	-	-	-	-	-	7	7	10	6	7	5	13	8	14	5	-	-	-	-	-	-
	PERCENT POSITIVE	-	-	-	-	-	-	-	-	-	-	100	100	80	50	14	0	8	0	7	0	-	-	-	-	-	-
												100			52				4								
	DEGREE OF CLINICAL AFFECTION OBSERVED IN EACH CHILD WITH MOTTLED ENAMEL. &											4,	4,	4,													
												4,	4,	3,													
												4,	4,	2,	4,												
												3,	3,	2,	1,	1,	-	1,	-	1,	-	-	-	-	-	-	-
												3,	2,	2,	1,												
												3,	2,	2,													
												2,	2,	1,													
														1,													

↓ INCIDENCE ENTIRE GROUP - 98 PERCENT
& 1: VERY MILD; 2: MILD; 3: MODERATE; 4: SEVERE.

FIGURE 2.—*Comparison of conditions in 1928 and 1938.*

PERIOD OF SUSCEPTIBILITY TO MOTTLED ENAMEL

In evaluating clinical data recorded in mottled enamel studies where the age distribution of the group examined overlaps a change in the common water supply, it is essential that the period of susceptibility to mottled enamel be clearly understood. Susceptibility to mottled enamel is limited to a sharply defined age group of the population, namely, those in whom the crowns of the permanent teeth are calcifying. The interval in time between the period during which the toxic fluorides are operative, and the time when the objective evidence is apparent, is unusually long.

In order that this period of susceptibility (period when crowns of the teeth are calcifying), the quiescent period (preeruptive), and the time when the pathology is evident (time of eruption) may be clearly understood, the table of the chronology of permanent human dentition by Kronfeld is reproduced here:

Chronology of the human dentition (permanent teeth)

[Logan and Kronfeld [1]]

Tooth			First evidence of calcification	Crown completed (years of age)	Eruption (years of age)	Root completed (years of age)
Permanent dentition.	Upper jaw..	Central incisor	3 to 4 months	4 - 5	7- 8	10
		Lateral incisor	1 year	4 - 5	8- 9	11
		Cuspid	4 to 5 months	6 - 7	11-12	13-15
		First bicuspid	1½ to 1¾ years	5 - 6	10-11	12-13
		Second bicuspid	2 to 2¼ years	6 - 7	10-12	12-14
		First molar	At birth	2½- 3	6- 7	9-10
		Second molar	2½ to 3 years	7 - 8	12-13	14-16
		Third molar	7 to 9 years	12 -16	17-21	18-25
	Lower jaw..	Central incisor	3 to 4 months	4 - 5	6- 7	9
		Lateral incisor	do	4 - 5	7- 8	10
		Cuspid	4 to 5 months	6 - 7	10-11	12-14
		First bicuspid	1¾ to 2 years	5 - 6	10-12	12-13
		Second bicuspid	2¼ to 2½ years	6 - 7	11-12	13-14
		First molar	At birth	2½- 3	6- 7	9-10
		Second molar	2½ to 3 years	7 - 8	12-13	14-15
		Third molar	8 to 10 years	12 -16	17-30	18-25

COMPARISON OF FINDINGS OF 1928 SURVEY AND THE 1938 SURVEY

The findings of the 1928 survey were fortunately recorded in a manner that facilitates comparison with the 1938 data. Due to the fact that certain groups of teeth calcify at different periods in the child's life, Kempf and McKay (*1*) divided the permanent teeth into three different groups, the divisions being based upon the order of calcification in order of time. Their grouping is as follows:

First group	*Second group*
First molars	Second bicuspids
Incisors	Cuspids
First bicuspids	Second molars

Third group

Third molars

In that portion of the school population showing continuous exposure to the Bauxite deep-well water, Kempf and McKay reported the incidence of mottled enamel by single age groupings with a further classification according to the chronological divisions of calcification just stated.

An analysis of the data from the 1938 survey shows that practically all (98 percent) of the permanent teeth erupted in the Bauxite children, ages 6 to 10, are teeth included in the "first group" (1st molars, incisors, and 1st bicuspids). Hence, in order to show the contrast between data apparently comparable, except for the important difference in the chemical character of the domestic water supply, the findings of the 1928 and 1938 surveys will be limited to a comparison of the changes noted in the permanent teeth in the "first group."

The report of the 1928 survey recorded the condition of the enamel in 66 children, ages 5 to 18, who, the authors state, were born in Bauxite, had lived there all their lives, and who had always used the deep-well water supply. In this group there were three children who at the time of the examination presented no erupted permanent teeth. These three will be eliminated from further consideration. Four others, aged 5, 16, 17, and 18, respectively, are likewise excluded in order that the age grouping of the 1928 and the 1938 surveys may be similar.

A comparison of the mottled enamel conditions of the 59 children examined in 1928 with those of 82 examined in 1938 is shown in figure 2.

An analysis of the data presented in figure 2 shows that mottled enamel was present in 58 of the 59 children examined in 1928. With regard to the one 6-year-old child listed as normal, Kempf and McKay record in a footnote as follows: "City water available in house but was not used for cooking and drinking."

Study of the 1938 survey shows that the 14 children, 14 and 15 years of age, whose "first group" of permanent teeth were calcified while using the deep-well water, each show mottled enamel, generally of a marked type. The 11-, 12-, and 13-year-old group obviously represents a group of children whose teeth were partially calcified under the influence of the "old" supply and partially while using the "new" supply. Of the ten 13-year-old children who had used the deep-well supply for 3 years prior to the change, eight were affected. Among the six 12-year-old children who had been exposed for 2 years prior to the change, three showed evidence of mottled enamel; but of the seven 11-year-old children who had used the old supply for only a year, only one showed mottled enamel, and that of "very mild" degree. Of the 45 children, ages 6 to 10, born about the time of or subsequent to the change in water supply, 43 showed no evidence of mottled enamel. The two cases diagnosed as positive were each "very mild." The classification "very mild" is used to designate the mildest type of mottled enamel, the white opacity which shows irregularly and involves not more than 25 percent of the tooth surface. In most cases, too, only a few teeth are affected, the remainder of the dentition being normal. The most frequent use of this classification is in those endemic areas where the fluoride content of the water supply is just above the minimal threshold, 1.0 part per million. In such places, the examiner may find 25 to 30 percent of the children showing the mildest form of dental fluorosis, the others generally being normal or questionable. Even in the surveys of places where the community mottled enamel index is negative (less than 10 percent of the children affected) sporadic cases of very mild degree are occasionally observed. Whether these very mild markings in a community whose index is negative suggest an unusual susceptibility on the part of the child to very small amounts of fluorides (<1.0 p.p.m. of F), a water intake markedly in excess of that common to the child's age group, peculiarities in culinary habits (high frequency of soups, stews, or boiled cereals, etc.) in the child's home, or other factors, is at present unknown.

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[1] The Bur, 35: 18-25 (March 1935).

In the case of Bauxite, it is well to remember that the water used by the residents during the first few years after the change to the filtered river water may have been slightly higher in fluoride content than the present supply. The incrustations in the iron pipes of the distribution system or in cooking utensils in the homes may have been sufficient to bring the fluoride (F) content of the water up to an amount which could produce sporadic cases of very mild mottled enamel in 4 to 5 percent of the continuous users.

Furthermore, it must be borne in mind that conditions in Bauxite were quite different from those in the average endemic area in the United States. Actually the population using the deep-well supply was ingesting about 5½ times as much fluorine as the residents of Colorado Springs and about 3½ times as much as the residents of Amarillo, Tex. The possibility, therefore, of skeletal storage and antepartum maternal fluorosis with subsequent transfer of small amount of fluorine in the maternal milk, must be given thoughtful consideration. Roholm (*3*) has recorded a "moderate" type of mottled enamel in three children nursed for a relatively long period (1 to 2 years) by mothers exposed to cryolite dust previous to gestation and lactation, the fluorine intoxication in the children apparently occurring through the mother's milk. The 7-year-old child at Bauxite in whom "very mild" mottled enamel was observed was nursed 15 months.

It may be observed that the daily use of a domestic water containing 13.7 p.p.m. of fluorine possibly results in a daily fluorine absorption equal to approximately half of that estimated for the cryolite workers who developed osteosclerosis as described by Roholm. Among the 43 children recorded as normal, there were 5 children showing slight aberrations in the enamel of a few of the permanent teeth, generally white flecks or spots. The abnormalities were of a questionable type and a positive diagnosis of even the mildest form of mottled enamel was not warranted.

MOTTLED ENAMEL IN RELATION TO TOTAL ERUPTED PERMANENT TEETH

Probably a better way to illustrate the marked changes resulting from the change in the communal water supply is to record the number of permanent teeth affected by mottled enamel in relation to the total number of erupted permanent teeth. Comparisons are limited to the permanent teeth of the "first group" in those 82 children whose continuity of exposure was verified by an interview with the child's parent.

The agreement between the two examiners with respect to mottled enamel diagnosis was so close that there would be little value in a detailed recording of their individual findings with respect to specific teeth. In order, therefore, to simplify the presentation of these data, the findings of one examiner only will be shown. These data are given in table 2.

TABLE 2.—*Percentage of erupted permanent teeth affected with mottled enamel in relation to the domestic water used during the period of calcification*

	First group of permanent teeth calcified while using—									
	Deep-well water		Both water supplies			Filtered river water				
Age of children examined	15	14	13	12	11	10	9	8	7	6
Number of children examined	7	7	10	6	7	5	13	8	14	5
Number of first molars, incisors, and first bicuspids present	112	108	152	93	108	67	157	85	121	31
Number affected with mottled enamel	95	83	73	22	2	0	[1] 3	0	[1] 4	0
Percentage of permanent teeth affected	84.8	76.8	48.0	23.4	1.8	0	1.9	0	3.3	0
Percentage, all ages	80.9		27.4			1.5				

[1] All teeth listed in this group as having mottled enamel were classified as "very mild." As a contrast in the marked quantitative differences in severity, only 36, or about 20 percent, of the 178 affected teeth in the 14-15 year age group were so slightly affected as to permit their inclusion in the lowest grade of mottled enamel classification, "very mild."

NOTE.—Erupting permanent teeth not showing approximately 50 percent of crown and teeth covered by food debris obviating adequate inspection excluded from above data.

THIRD MOLAR IMMUNITY FROM MOTTLED ENAMEL IN HIGHER AGE GROUPS

In surveying endemic areas it is a common experience to note among certain older high school pupils individuals with all permanent teeth showing normal calcification with the exception of the third molars, which are mottled. This condition is seen in children who take up residence in an endemic area when about 8 to 10 years of age and use the mottled-enamel-producing water during the period of third molar calcification.

It was interesting to note a complete reversal of this common observation in the study of Bauxite. Seven or eight young men and women in the Senior High School were examined, some of whom had also been examined in the 1928 survey.

Practically all permanent teeth with the exception of the third molars were severely affected by mottled enamel. But the third molars, calcified at a later date (since the change to the filtered river water), showed normal calcification. The repeated observation of 4 normally calcified third molars in an individual with the other 28 permanent teeth severely marked by mottled enamel furnished a striking illustration of the effects resulting from the change in the water supply.

The photograph shown in figure 4 was taken during the 1928 survey. A reexamination of this person in 1938 showed the condition just described—4 normally calcified third molars, with the other 28 permanent teeth severely affected by mottled enamel.

CHEMICAL ANALYSES OF WATERS USED

As has been our custom in other mottled enamel surveys, analyses were made of constituents of the water other than flourides. The fluoride content was estimated colorimetrically by means of the zirconium-alizarin reagent (*4*). Results of the chemical analyses of the waters are given in table 3, the chemical findings made in connection with the 1928 study (*1*) being included for comparative purposes.

DISCUSSION

The development of the endemic hypoplasia known as chronic endemic dental fluorosis (mottled enamel) has again been halted by changing from a common water supply containing toxic amounts of fluorides to one relatively low in fluorides. One of us (F. S. McK.) has previously described (*5*) a similar result in an experiment at Oakley, Idaho, the first known instance of a community abandoning an otherwise satisfactory common water supply solely for the purpose of preventing the development of mottled enamel.

On the basis of the degree of affection observed in the 14- and 15-year-old children whose "first group" of permanent teeth were calcified largely under the influence of the "old" deep-well supply, the tentative community mottled enamel index associated with the deep-well supply was "very marked." The application of this same method (*6*) to the percentage distribution of severity in the 45 children born since the change in the water supply shows that the "actual mottled enamel index" of Bauxite at present is "negative."

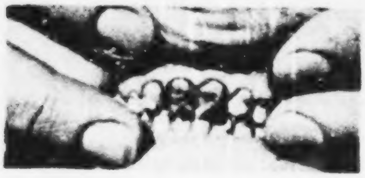

FIGURE 3.—*Severe (confluent pitting).*

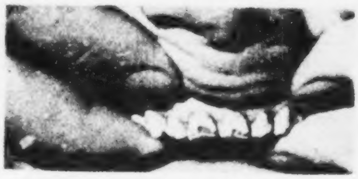

FIGURE 4.—*Severe (confluent pitting).*

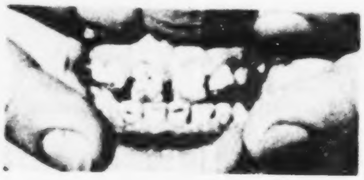

FIGURE 5.—*Severe (discrete pitting).*

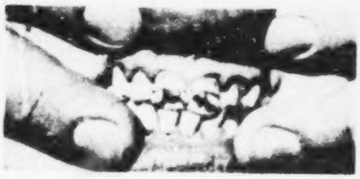

FIGURE 6.—*Moderate (brown stain).*

Teeth of children in whom the calcification of the permanent teeth occurred while using the deep-well water supply. (Photographs taken during the 1928 survey.)

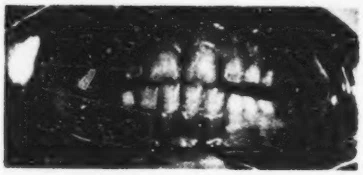

FIGURE 7.—*Normal.*

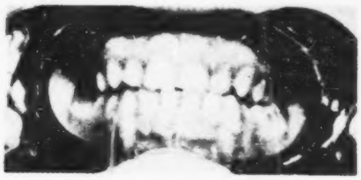

FIGURE 8.—*Normal.*

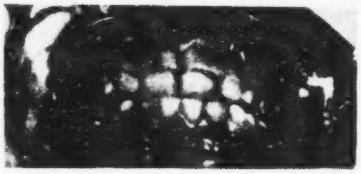

FIGURE 9.—*Normal.*

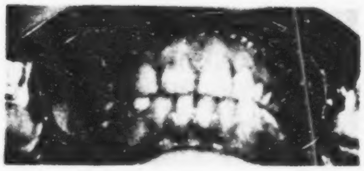

FIGURE 10.—*Normal.*

Teeth of children in whom the calcification of the permanent teeth occurred while using the filtered river water exclusively

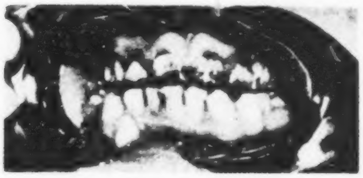

FIGURE 11.—*Moderate (brown stain).*

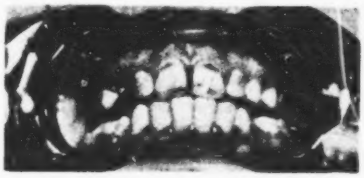

FIGURE 12.—*Mild.*

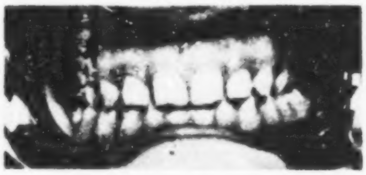

FIGURE 13.—*Very mild.*

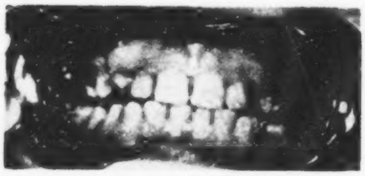

FIGURE 14.—*Very mild.*

Teeth of children in whom the calcification of the permanent teeth occurred while using the deep-well supply in varying amounts.

TABLE 3.—*Analyses of the waters used*

	1928 [1]		1938 [2]			
	Bauxite deep-well water	Bauxite filtered water	Bauxite 245-foot deep well	Bauxite filtered river water	247-foot Norton Town well	Hamp-Smith spring
	p. p. m.	*p. p. m.*	*p. p. m.*	*p. p. m.*	*p. p. m.*	*p. p. m.*
Total residue on evaporation	1,003.0	86.0	958.6	60.0	805.3	23.8
Loss on ignition	43.0	14.0	27.4	10.6	26.9	4.4
Fixed residue	960.0	72.0	931.2	49.4	778.4	19.4
Silica (SiO_2)	18.6	6.0	13.6	5.8	9.0	10.4
Iron (Fe)	1.0	.3	.1	.02	.09	.02
Aluminum (Al)			.4	0	0	0
Calcium (Ca)	25.3	17.6	30.9	11.4	17.7	1.8
Magnesium (Mg)	7.0	2.1	8.8	3.7	6.6	0
Sodium and potassium (calculated as Na)	----------	----------	333.4	2.9	287.7	2.4
Sodium (Na)	344.6	9.6	----------	----------	----------	----------
Potassium (K)	9.2	3.4	----------	----------	----------	----------
Carbonate (CO_3)	1.2	0	0	0	4.8	0
Bicarbonate (HCO_3)	258.2	63.4	247.6	47.5	287.9	4.9
Sulfate (SO_4)	39.6	15.7	36.0	10.3	25.3	2.2
Nitrate (NO_3)	.3	.03	0	.27	0	.13
Chloride (Cl)	415.9	3.75	389.5	3.0	274.5	2.0
Fluoride (F)	([3])	----------	14.1	0	16.9	0
Phosphate (PO_4)	----------	----------	.1	0	.1	0
Boron (B)	----------	----------	.8	0	.8	0

[1] Samples collected July 1928.

[2] Samples collected in March 1938. Assistant Chemist C. G. Remsburg carried out the determinations other than fluoride and boron, using mostly the methods given in the Standard Methods of Water Analysis of the American Public Health Association. The phosphate was determined colorimetrically by an adaptation of the Benedict and Theis method (J. Biol. Chem., 61: 63 (1924)). The boron determinations were made essentially by the method of Foote (J. Ind. Eng. Chem., Anal. Ed., 4: 39 (Jan. 15, 1932)).

[3] In 1931 Churchill reported 13.7 p. p. m. of F in the Bauxite deep well.

SUMMARY

1. The production of an unusually severe type of endemic dental flourosis (mottled enamel) at Bauxite, Ark., was halted with the change in the common water supply.

2. This is the second recorded instance in the United States where a community has abandoned the use of an otherwise satisfactory common water supply solely for the purpose of preventing the development of permanent dental disfigurements among its children. The efforts in each instance were successful.

ACKNOWLEDGMENTS

The writers are indebted to Chief Sanitary Engineer F. L. McDonald and Assistant Sanitary Engineer Walter A. Reinman, Arkansas State Board of Health, to Dr. J. Scott Walker, Chetopa, Kans., and to company officials of the Republic Mining and Manufacturing Company for their assistance and cooperation in the study at Bauxite, and to Senior Statistician Wm. M. Gafafer, National Institute of Health, for helpful suggestions relative to presenting the data shown in figure 2.

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(*4*) Elvove, E.: Estimation of fluorides in waters. Pub. Health Rep., *48*: 1219–1222 (Oct. 6, 1933). (Reprint No. 1596.)

(*5*) McKay, F. S.: Mottled enamel: The prevention of its further production through a change of the water supply at Oakley, Idaho. J. M. Dent. Assoc., *20*: 1137–1149 (July 1933).

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MOTTLED ENAMEL IN SOUTH DAKOTA

By H. TRENDLEY DEAN, ELIAS ELVOVE, and RICHARD F. POSTON

Mottled enamel has been endemic in South Dakota for a number of years. As early as 1916 McKay (*1*) demonstrated by surveys its presence at Kidder, Britton, Langford, Pierpont, Andover, Groton, and Aberdeen. In each of these communities the endemicity was associated with the use of artesian well water obtained from the Dakota sandstone.

In 1932 (*2*) one of us (HTD), while studying selenium poisoning in South Dakota, observed endemic mottled enamel at Wolsey, Vayland, St. Lawrence, Miller, and at the Spear's School midway between Miller and Ree Heights. Evidence of mottled enamel was also noted in children living south and east of Harrold and in the rural districts north of Holabird and Highmore. A mild degree of mottled enamel was also observed in children residing on ranches in the northwest section of Lyman county and a border line degree of affection was present at Edgemont, apparently associated with the common water supply. Time did not permit detailed studies, but the development of mottled enamel in each locality was seemingly associated with the use of deep well water.

In the questionnaire study of geographical distribution of mottled enamel conducted by the United States Public Health Service and published in 1933 (*2*), questionnaires were sent to 142 selected South Dakota dentists, of whom 78 replied. Their answers pointed to numerous other localities which were listed in the 1933 report as "reported" mottled enamel areas. These places included Cavour, Yale, Iroquois, Hitchcock, Chelsea, Lake Preston, Bancroft, Fedora, Roswell, Vilas, Carthage, Esmond, the Whetstone Valley of Roberts County, Artesian, Forestburg, Doland, Conde, and Frankfort.

METHOD OF SURVEY

The present study was made by the United States Public Health Service with

Table 1.—*Summary of mottled enamel findings and history of water supply in certain cities and towns of eastern South Dakota*

Town and population (Census of 1930)	Total number of children examined	Children classified according to mottled enamel diagnosis						History of water supply	Remarks
		Normal	Questionable	Very mild	Mild	Moderate	Severe		
ST. LAWRENCE (413)									
(a) Continuous residence	62	5	2	6	6	9	0	From 1 2-inch 1,300-foot well installed in 1920.	All children 3d to 8th grade, inclusive, were examined, plus 1st and 2d grades and high-school pupils with continuous exposure.
(b) Nearby rural		4	0	0	2	1	0		
(c) Discontinuities		14	2	6	2	3	0		
PIERPONT (379)									
(a) Continuous residence	69	1	2	7	9	7	0	From 1 6-inch 1,165-foot well drilled in 1912 and from 1 6-inch 90-foot well. The shallow well furnishes only about 1 percent of the town's water.	All children 3d to 8th grade, inclusive, were examined.
(b) Nearby rural		6	4	2	0	3	0		
(c) Discontinuities		15	3	4	2	4	0		
WOLSEY (455)									
(a) Continuous residence	90	1	3	2	6	4	0	From 1 4-inch 980-foot well installed in 1920.	All children 3d to 12th grade, inclusive, with continuous exposure in Wolsey or rural district were examined. (c) Includes children from 3d to 8th grades only.
(b) Nearby rural		37	3	3	1	1	0		
(c) Discontinuities		20	0	4	5	0	0		
PLATTE (1,207)									
(a) Continuous residence	75	9	10	12	12	10	2	From 2 wells, 1 6-inch 465-foot installed in 1914 and from an 8-inch 850-foot well installed in 1936. A 6-inch 465-foot well constructed in 1924 was abandoned in 1926 and an 8-inch 290-foot well drilled in 1926 was abandoned in 1936.	All children 3d to 12th grade, inclusive, with continuous residence were examined.
(b) Discontinuities		14	3	2	1	0	0		
HITCHCOCK (334)									
(a) Continuous residence	40	2	2	5	2	3	0	Between 1910 and 1937 from a 3-inch, 900-foot well. Since 1937 from a 1½-inch, 1,040-foot well.	All children 4th to 8th grade, inclusive, examined.
(b) Nearby rural		8	1	0	0	0	0		
(c) Discontinuities		9	2	3	2	1	0		
REDFIELD (2664)									
(a) Continuous residence	117	14	5	14	5	9	1	From 4 wells; 2 4½-inch drilled in 1906 and 1911, respectively; 1 6-inch drilled in 1917 and 1 8-inch drilled in 1920. A 3-inch well drilled in 1908 and a 6-inch well drilled in 1914 were abandoned in 1933. All wells approximately 1,000 feet deep.	All children of the 4th, 5th, and 6th grades were examined.
(b) Discontinuities		48	3	12	2	4	0		
IROQUOIS (531)									
(a) Continuous residence	99	8	2	8	5	4	1	Present supply obtained from 2 6-inch, 900-foot wells installed in 1900 and 1923. Between 1908 and 1937 the supply was augmented with water from a 2½-inch 900-foot well, now abandoned.	All children 4th to 12th grade, inclusive, were examined.
(b) Nearby rural		8	0	4	3	2	0		
(c) Discontinuities		30	5	10	7	2	0		

the active cooperation and assistance of the South Dakota State Board of Health. The planning of the itinerary was facilitated by the extensive preliminary chemical studies of common water supplies made by the division of sanitary engineering of the State board of health, in cooperation with the State chemist.

Each of the communities hereinafter referred to was visited, and subsequently with the cooperation of the local superintendent of education, school children, generally of the third to the eighth grades, inclusive, were examined. A total of 53 cities, towns, or rural communities in 21 counties was visited and 3,350 school children were examined, including 15 children examined at Harrold and 8 at Bristol but not included in tables that follow, and the 35 examined at Andover which are discussed separately in the text. The survey provided general information on the extent of the affected territory and an approximate index of the degree of severity of the mottled enamel being produced.

Upon visiting a classroom, the purpose of the survey was first explained and those children who stated that they had lived in the community continuously since birth and had always used the common water supply for domestic purposes (drinking and cooking) were assembled in a separate group. This group was then further questioned to determine whether there had been any breaks in the continuity of their residence and water consumption. If questioning disclosed breaks in the continuity of exposure (30 days in any calendar year excepted) the child was eliminated from this group. This group in the tables to follow is listed as "(*a*) Continuous residence." The remaining pupils in the classroom were then divided into two groups—those who had always lived in the surrounding rural district (boundaries of the school district) and shown in the tables under the heading "(*b*) Nearby rural," and those with two or more residences and water supplies and shown in the tables as "(*c*) Discontinuities." With the pupils divided into three groups, under good illumination each child was examined by one of us (HTD) and the presence or absence of mottled enamel recorded, the degree of severity being noted in accordance with a standard of classification previously described (*3*). This classification of diagnosis has since been abridged by combining "Moderately Severe" and "Severe" into one classification: "Severe."

The examination of the first group indicated the degree of endemicity associated with the use of the communal water supply; the examination of the second group showed whether or not the types of water used on the nearby farms were producing mottled enamel; while the examination of the third group, those with different residences and water histories, frequently revealed clinical mottled enamel developed either in other areas of known endemicity or in localities hitherto unreported as endemic areas.

SURVEY FINDINGS

The results of this survey are summarized as follows: Table 1 details the mottled enamel findings and history of the common water supply of 37 communities where a sufficient number of children were examined to warrant the computation of an approximate or tentative community mottled enamel index. Figure 1 shows the percentage distribution according to severity of affection of that part of the group examined who stated that they had resided continuously in the community and had always used the common water supply. In addition the percentage incidence of affection and the approximate or tentative mottled enamel index of the community is listed.

In some instances the fluoride (F) content of the common water supply at the time of the clinical examination is also given. Extreme caution, however, should be followed in correlating many of these chemical findings with the clinical observations. Because of inadequateness of the sample in certain communities or the frequency with which new wells were added or old ones abandoned during the lifetime of those examined, certain of these communities lack the requisites for a quantitative evaluation (*4*).

In table 2 is listed the summary of mottled enamel findings in certain communities having a common water supply but where an insufficient number of children were examined to permit the computation of even a tentative community mottled enamel index. Table 3 summarizes the findings in six communities, four of which have no common water supply and two where the mineral composition of the common water supply is such that the water is not used for domestic purposes.

The geographical distribution of mottled enamel is shown in figure 2, the indexes being computed upon the basis of the percentage distribution of clinical severity in the manner previously cited (*3*).

Clinical evidence suggestive of other endemic areas.—During the examinations in the school, numerous children were examined who had lived in two or more places. In tables 1, 2, and 3 such children are listed under the heading "(c) Discontinuities." In this group were many children who presented clinical evidence of mottled enamel. Cross questioning often revealed residence during the susceptible period in a known endemic area. There were, however, other children showing clinical signs of mottled enamel, who stated that they had resided during the susceptible period in areas not as yet surveyed. The actual observation of clinical mottled enamel from these places warrants their listing as "probable" endemic areas pending confirmation by surveys. These places are:

County	*Towns or Rural Districts*
Aurora	Stickney and northern part of county.
Beadle	Yale, Virgil.
Brown	Putney, Ferney, Bath, and rural districts north of Aberdeen.
Edmunds	Craven, Mina.
Faulk	Chelsea, Cresbard, Orient.
Hamlin	Hazel.
Kingsbury	Bancroft, Hetland.
Marshall	Newark, Amherst.
McPherson ...	Wetonka.
Perkins	Lemmon.
Potter	Gorman.
Sanborn	Northeast and western section of county.
Spink	Athol, Crandon, Turton.
Turner	Parker.
Union-Lincoln.	Beresford.*

* A recent survey by Dr. R. H. Wilcox, epidemiologist, State Board of Health, has demonstrated endemic mottled enamel at Beresford.

As has been previously noted, endemic mottled enamel has also been observed in children residing in the rural districts north of Holabird and Highmore.

Effects following the change of water supply at Andover, South Dakota.—The previously mentioned survey by McKay in 1916 (*1*) disclosed endemic mottled enamel at Andover associated with the use of the town artesian well water. In 1928 this community changed its

TABLE 1.—*Summary of mottled enamel findings and history of water supply in certain cities and towns of eastern South Dakota*—Continued

Town and population (Census of 1930)	Total number of children examined	Children classified according to mottled enamel diagnosis						History of water supply	Remarks
		Normal	Questionable	Very mild	Mild	Moderate	Severe		
ST. LAWRENCE (413)									
(a) Continuous residence.	62	5	2	6	6	9	0	From 1 2-inch 1,300-foot well installed in 1920.	All children 3d to 8th grade, inclusive, were examined, plus 1st and 2d grades and high-school pupils with continuous exposure.
(b) Nearby rural		4	0	0	2	1	0		
(c) Discontinuities		14	2	6	2	3	0		
PIERPONT (379)									
(a) Continuous residence.	69	1	2	7	9	7	0	From 1 6-inch 1,165-foot well drilled in 1912 and from 1 6-inch 90-foot well. The shallow well furnishes only about 1 percent of the town's water.	All children 3d to 8th grade, inclusive, were examined.
(b) Nearby rural		6	4	2	0	3	0		
(c) Discontinuities		15	3	4	2	4	0		
WOLSEY (455)									
(a) Continuous residence.	90	1	3	2	6	4	0	From 1 4-inch 980-foot well installed in 1920.	All children 3d to 12th grade, inclusive, with continuous exposure in Wolsey or rural district were examined. (*c*) Includes children from 3d to 8th grades only.
(b) Nearby rural		37	3	3	1	1	0		
(c) Discontinuities		20	0	4	5	0	0		
PLATTE (1,207)									
(a) Continuous residence.	75	9	10	12	12	10	2	From 2 wells, 1 6-inch 465-foot installed in 1914 and from an 8-inch 850-foot well installed in 1936. A 6-inch 465-foot well constructed in 1924 was abandoned in 1926 and an 8-inch 290-foot well drilled in 1926 was abandoned in 1936.	All children 3d to 12th grade, inclusive, with continuous residence were examined.
(b) Discontinuities		14	3	2	1	0	0		
HITCHCOCK (234)									
(a) Continuous residence.	40	2	2	5	2	3	0	Between 1910 and 1937 from a 3-inch, 900-foot well. Since 1937 from a 1½-inch, 1,040-foot well.	All children 4th to 8th grade, inclusive, examined.
(b) Nearby rural		8	1	0	0	0	0		
(c) Discontinuities		9	2	3	2	1	0		
REDFIELD (2664)									
(a) Continuous residence.	117	14	5	14	5	9	1	From 4 wells; 2 4½-inch drilled in 1906 and 1911, respectively; 1 6-inch drilled in 1917 and 1 8-inch drilled in 1920. A 3-inch well drilled in 1908 and a 6-inch well drilled in 1914 were abandoned in 1933. All wells approximately 1,000 feet deep.	All children of the 4th, 5th, and 6th grades were examined.
(b) Discontinuities		48	3	12	2	4	0		
IROQUOIS (531)									
(a) Continuous residence.	99	8	2	8	5	4	1	Present supply obtained from 2 6-inch, 900-foot wells installed in 1900 and 1923. Between 1908 and 1937 the supply was augmented with water from a 2½-inch 900-foot well, now abandoned.	All children 4th to 12th grade, inclusive, were examined.
(b) Nearby rural		8	0	4	3	2	0		
(c) Discontinuities		30	5	10	7	2	0		

TABLE 1.—*Summary of mottled enamel findings and history of water supply in certain cities and towns of eastern South Dakota*—Continued

Town and population (Census of 1930)	Total number of children examined	Children classified according to mottled enamel diagnosis: Normal	Questionable	Very mild	Mild	Moderate	Severe	History of water supply	Remarks
DOLAND (538)									
(a) Continuous residence	54	5	4	2	10	4	0	From 1 6-inch and 1 3-inch well drilled in 1925 and 1935, respectively. Both wells approximately 1,000 feet deep.	All children 3d to 8th grade, inclusive, were examined.
(b) Nearby rural		3	3	1	1	1	0		
(c) Discontinuities		3	3	7	3	4	0		
MELLETTE (363)									
(a) Continuous residence	42	5	4	4	6	2	1	From 2 2-inch, 930-foot wells installed in 1910.	All children 4th to 8th grade, inclusive, were examined, plus all high-school pupils with continuous exposure.
(b) Discontinuities		8	3	4	3	1	1		
IPSWICH (913)									
(a) Continuous residence	99	11	9	15	12	5	1	From 3 2-inch wells, 1,200 feet, 1,470 feet, and 1,540 feet deep, respectively. Date of installation unknown.	All children 3d to 12th grade with continuous exposure examined; in addition, rural children, 3d to 6th grade and broken histories, 3d to 8th grade.
(b) Nearby rural		6	0	1	1	0	0		
(c) Discontinuities		21	5	7	5	0	0		
GETTYSBURG (1,400)									
(a) Continuous residence	151	17	7	16	8	3	2	From 1 1½-inch, 2,130-foot well drilled in 1900 and from a 4-inch, 1,920-foot well installed in 1922.	All children 3d to 8th grade with continuous exposure examined. Rural and broken histories from 4th to 8th grade, inclusive.
(b) Nearby rural		15	1	2	0	0	0		
(c) Discontinuities		70	3	1	5	1	0		
FAULKTON (730)									
(a) Continuous residence	96	15	5	24	7	5	0	From a 3-inch, 1,400-foot well drilled in 1922. Prior 1922 from a well similar in diameter and depth abandoned in 1926.	All children 3d to 8th grade, inclusive, were examined.
(b) Nearby rural		2	0	0	0	0	0		
(c) Discontinuities		23	2	6	3	4	0		
MILLER (1,447)									
(a) Continuous residence	105	17	9	15	7	3	0	From a 1,650-foot, 6-inch well installed in 1926. Prior to that time from 3 4-inch, 1,650-foot wells directly connected to the mains. These wells are at present connected with the mains but due to lowered artesian pressure furnish but a small amount of water. Six-inch well is pumped.	All children 4th to 7th grade examined, plus 8th grade pupils with continuous exposure.
(b) Nearby rural		6	0	0	1	0	0		
(c) Discontinuities		29	2	11	2	3	0		
CARTHAGE (500)									
(a) Continuous residence	77	9	7	8	8	2	0	From 1 4-inch, 1,100-foot well installed in 1906.	All children 3d to 8th grade examined, plus high school pupils with continuous exposure.
(b) Nearby rural		2	0	0	3	0	0		
(c) Discontinuities		12	3	9	5	8	1		
FEDORA (225)									
(a) Continuous residence	67	8	2	4	2	1	0	Communal supply. Town is supplied by groups of individuals sharing the expense of drilling and piping flowing artesian wells. Wells are all 1 inch or 1¼ inches in diameter and are approximately 700 feet deep.	All children examined, 4th to 8th grade, inclusive, plus high school pupils of continuous exposure in both Fedora and surrounding rural districts.
(b) Nearby rural		9	9	8	3	4	0		
(c) Discontinuities		5	4	7	1	0	0		

common water supply from the 800-foot artesian well to a dug shallow well 22 feet deep. There is a local record that the supply from the 800-foot artesian well was augmented between 1926 and 1928 by a small amount of water from a 170-foot well. Both the 800-foot well and the 170-foot well were abandoned in 1928, and since that date the common water supply of Andover has been obtained wholly from the dug shallow well.

This survey revealed the marked clinical difference resulting from the change in the common water supply. In the Andover school were 35 children who stated that they were born in Andover, had resided there continuously, and had always used the common water supply. Fourteen of this group were between 7 and 10 years of age, a group that obviously used the shallow well water exclusively for domestic purposes. None of the 14 showed evidence of mottled enamel. In the intermediate group there were 8 children, ages 11 to 13, who had calcified their permanent teeth while using both water supplies, and 3 of the 8, or 37 percent, showed mottled enamel; and in the highest age group, 14 to 18, there were 13 children who apparently calcified their teeth while using the artesian water, and 9 of these, or 69 percent, showed mottled enamel. These differences are presented in tabular form in table 4.

Water supplies.—Endemic mottled enamel in South Dakota seems limited to the users of artesian water obtained from the Dakota sandstone. For a complete description of this aquifer the reader is referred to the work of Darton (Water Supply Paper No. 227, United States Geological Survey, and earlier publications). Data respecting the common water supplies referred to in the tables under the heading "History of Water Supplies" were obtained by one of the authors (RFP).

During the survey, samples of the present common water supply of 18 communities and 2 samples of the "old" Aberdeen supply were collected for chemical analyses. The mineral composition of the samples, Aberdeen excepted, indicated merely the type of domestic water used at the time of the survey; it must be remembered that the clinical effects noted at the same time reflect the type of water used 8 to 15 years previously, dependent upon the age group examined. Reference to the data recorded in the tables under "History of Water Supplies" will reveal whether or not a correlation of the fluoride (F) content of the water with the clinical condition observed is warranted.

The fluoride content was estimated colorimetrically by means of the zirconium-alizarin reagent (*5*). In accordance with our usual custom, constituents of the water, other than fluoride, were likewise determined. These analyses are shown in table 5.

DISCUSSION

On the basis of studies to date endemic mottled enamel in South Dakota seems limited solely to users of artesian water obtained from the Dakota sandstone. Fortunately none of the larger cities in eastern South Dakota (Sioux Falls, Aberdeen, Huron, Watertown, or Mitchell) obtain their common water supplies from this aquifer and the endemicity is limited to the smaller communities and rural districts.

Chronic endemic dental fluorosis (mottled enamel) is widely distributed in South Dakota. The known endemic and "probable" areas with few exceptions are in that part of the State lying east of the Missouri River; together they embrace a total of 26 counties. Briefly the data presented in the distribution map shows that endemic mottled enamel has been demonstrated in 41 communities divided among 20 counties. In addition, a "border-line" degree of endemicity was observed in five communities. Furthermore, there are 30 other places listed as "probable" endemic areas on the basis of observed clinical mottled enamel in children who resided in these places during the period of susceptibility. Thirteen other communities either included in this survey or subject to observations made in 1932 are listed as "negative."

Although the Andover studies were only incidental to the main investigation, the facts disclosed deserve further attention. This community represents the third recorded instance of mottled enamel being prevented simply by changing the water supply from one containing comparatively high concentrations of fluorides to one that is free, or nearly free, from fluorides. The difficulty of obtaining evidence of this nature in a human population is evident when it is realized that a lapse of 8 to 10 years is required after the change in the water supply before the clinical consequence is demonstrable. The various aspects of the evidence of clinical differences, together with the report of the results noted in two other communities, Oakley, Idaho, and Bauxite, Arkansas, have been discussed in detail in another paper (*6*).

SUMMARY

1. There are 41 communities in South Dakota divided among 20 counties where endemic mottled enamel has been demonstrated by survey.
2. In addition there are 30 other places where mottled enamel is probably endemic.
3. The endemicity is seemingly limited to the smaller communities and rural districts where the inhabitants obtain their domestic water from the Dakota sandstone.

ACKNOWLEDGMENT

The authors are greatly indebted to State Sanitary Engineer W. W. Towne for his assistance in planning the study and providing much preliminary information on the fluorine content of numerous South Dakota public water supplies. This basic information permitted the outlining of an itinerary that resulted in considerably enlarging the region of known endemicity in South Dakota.

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TABLE 1.—*Summary of mottled enamel findings and history of water supply in certain cities and towns of eastern South Dakota*—Continued

Town and population (Census of 1930)	Total number of children examined	Children classified according to mottled enamel diagnosis						History of water supply	Remarks
		Normal	Questionable	Very mild	Mild	Moderate	Severe		
KIMBALL (111)									
(*a*) Continuous reisdence	58	24	4	6	5	1	1	From 1 4½-inch, 1,300-foot well installed in 1915.	All children of continuous exposure 4th to 12th grade, examined. Broken-history group from 7th grade only.
(*b*) Discontinuities		14	2	1	0	0	0		
ABERDEEN (16,465)									
(*a*) Continuous residence	166	109	20	24	10	3	0	At present from impounded surface (river) supply installed in 1935. Previously from 4 6-inch and 2 8-inch wells. Water from 3 of the wells emptied into covered reservoir southeast section of city; water from 2 wells emptied into open reservoir in northwest part of city: water from each reservoir pumped directly into distribution system. Water from sixth well pumped directly into distribution system. In addition there are reports of other artesian wells (number unknown) connected directly to the distribution system. Wells approximately 1,200 feet deep.	All children of continuous exposure in 4th, 5th, and 6th grades of 3 schools plus pupils of 7th and 8th grades of 1 school examined.
TULARE (205)									
(*a*) Continuous residence	44	2	1	4	3	0	0	From 1 3-inch 1,200-foot well installed in 1916.	All children 4th to 8th grade, inclusive, examined.
(*b*) Nearby rural		8	2	1	1	1	0		
(*c*) Discontinuities		15	3	3	0	0	0		
LEOLA (724)								From 1 4-inch 1,600-foot well installed in 1930. Prior to that year from a 1-inch 1,636-foot well installed in 1911 and abandoned in 1930.	Do.
(*a*) Continuous residence	103	9	6	10	6	0	0		
(*b*) Nearby rural		19	1	1	0	1	0		
(*c*) Discontinuities		37	6	6	1	0	0		
ONIDA (686)								From 2 wells. One 2½-inch 1,640-foot installed in 1911 and 1 6-inch 1,700-foot well put in in 1933.	All children 3d to 8th grade, inclusive, examined.
(*a*) Continuous residence	75	17	3	6	5	0	0		
(*b*) Nearby rural		11	0	0	0	1	0		
(*c*) Discontinuities		25	1	5	1	0	0		
FRANKFORT (367)								From 2 2-inch 1,000-foot wells installed in 1926 and from 1 3-inch 1,020-foot well drilled in 1929.	Do.
(*a*) Continuous residence	59	9	5	3	2	0	0		
(*b*) Nearby rural		2	3	6	1	1	0		
(*c*) Discontinuities		17	2	1	6	1	0		
GROTON (1,909)								From 2 wells, a 4-inch 900-foot well installed in 1927, owned by the city, and a 6-inch 900-foot well owned by C. M. St. P. and P. R. R. Most of common water supply from city well.	All children 4th to 8th grade, inclusive, examined.
(*a*) Continuous residence	104	11	5	13	2	0	0		
(*b*) Nearby rural		4	2	2	4	0	1		
(*c*) Discontinuities		28	5	11	7	6	3		

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(5) Elvove, E.: Estimation of fluorides in waters. Pub. Health Rep., *48*: 1219–1222 (Oct. 6, 1933). (Reprint No. 1596).

(6) Dean, H. T., and McKay, F. S.: Production of mottled enamel halted by a change in the common water supply. (Paper read before the Epidemiology Section of the American Public Health Association at the sixty-seventh annual meeting in Kansas City, Mo., Oct. 25, 1938).

TABLE 1.—*Summary of mottled enamel findings and history of water supply in certain cities and towns of eastern South Dakota*—Continued

Town and population (Census of 1930)	Total number of children examined	Children classified according to mottled enamel diagnosis						History of water supply	Remarks
		Normal	Questionable	Very mild	Mild	Moderate	Severe		
HOWARD (1,224)									
(a) Continuous residence	111	31	8	6	3	0	0	From 2 8-inch 405-foot wells drilled in 1900 and 1910 and still in use.	All children 4th to 8th grade, inclusive, examined, plus high-school pupils of constant exposure.
(b) Nearby rural		8	0	0	1	0	0		
(c) Discontinuities		38	5	9	0	2	0		
ASHTON (314)									
(a) Continuous residence	38	8	1	8	1	0	0	From 2 2-inch 1,100-foot wells installed in 1915 and 1929, respectively.	All children 3d to 8th grade, inclusive, examined.
(b) Nearby rural		0	0	1	1	0	0		
(c) Discontinuities		9	3	4	2	0	0		
CAVOUR (202)									
(a) Continuous residence	78	13	5	4	0	0	0	From 1 2-inch 850-foot well drilled in 1924. Prior that date from a well of same depth abandoned in 1926.	All children 1st to 12th grade, inclusive, examined.
(b) Nearby rural		20	2	3	1	1	0		
(c) Discontinuities		19	4	2	1	3	0		
ARLINGTON (1,020)									
(a) Continuous residence	47	40	6	1	0	0	0	From 2 wells, 2½ inches and 4 inches in diameter, 1,320 feet deep and installed in 1915 and 1930, respectively. 1915 well used for reserve purposes since 1930.	All children 4th to 12th grade of continuous exposure examined.
DE SMET (1,017)									
(a) Continuous residence	75	25	2	0	0	[illegible]	0	From a 42-foot gravel packed "Kelly Well" installed in 1927. Prior that date from a shallow well of same depth.	All children of constant residence, 4th to 12th grade, city and rural, examined. Broken histories from 8th grade only.
(b) Nearby rural		26	0	1	0	2	0		
(c) Discontinuities		14	3	2	0	0	0		
ALPENA (499)									
(a) Continuous residence	58	13	0	0	0	0	0	From 1 20-foot dug well 12 feet in diameter installed in 1920 and now used for reserve, and 2 4-inch flowing artesian wells, one 887 feet deep, installed in 1927, and one 784 feet deep, installed in 1935.	All children 3d to 8th grade, inclusive, examined.
(b) Nearby rural		16	1	0	0	0	0		
(c) Discontinuities		22	4	2	0	0	0		
WESSINGTON (681)									
(a) Continuous residence	76	21	0	0	0	0	0	Present supply from gravel packed 32-foot shallow well installed in 1936. Prior to 1936 from 2 30-foot dug wells put in in 1920 and 1928 and from 1 2½-inch 1,100-foot drilled well installed in 1903.	Do.
(b) Nearby rural		16	0	0	0	0	0		
(c) Discontinuities		33	3	2	1	0	0		
Total	2,941								

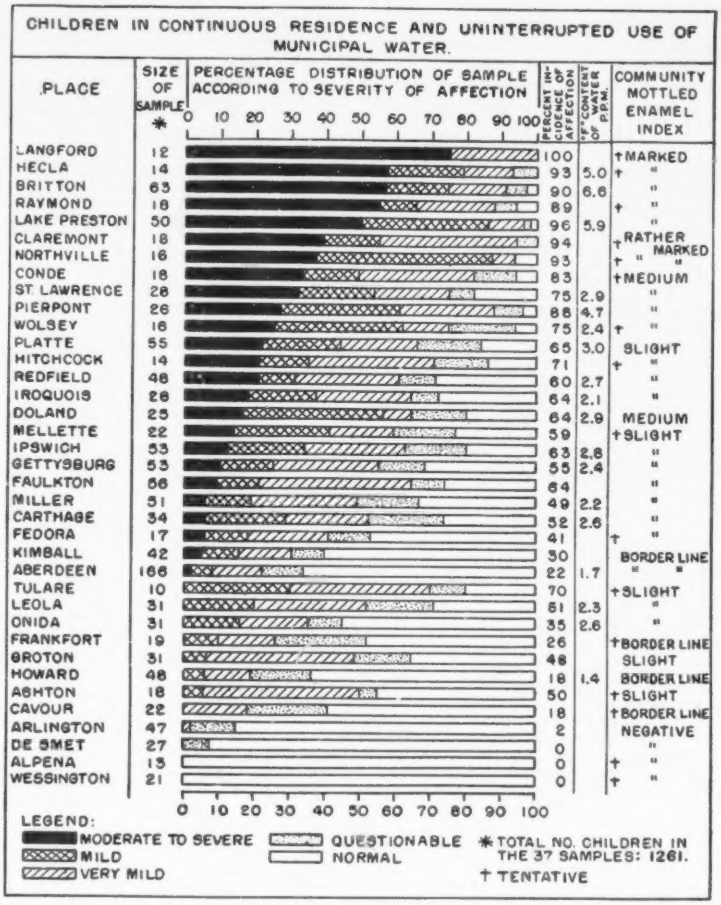

FIGURE 1.—Severity of mottled enamel in children of certain selected communities of eastern South Dakota.

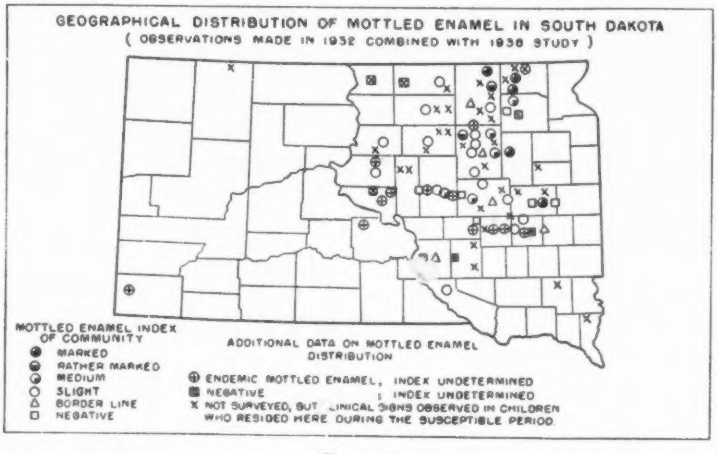
GEOGRAPHICAL DISTRIBUTION OF MOTTLED ENAMEL IN SOUTH DAKOTA
(OBSERVATIONS MADE IN 1932 COMBINED WITH 1936 STUDY)
MOTTLED ENAMEL INDEX OF COMMUNITY
MARKED
RATHER MARKED
MEDIUM
SLIGHT
BORDER LINE
NEGATIVE
ADDITIONAL DATA ON MOTTLED ENAMEL DISTRIBUTION
ENDEMIC MOTTLED ENAMEL, INDEX UNDETERMINED
NEGATIVE, INDEX UNDETERMINED
X NOT SURVEYED, BUT CLINICAL SIGNS OBSERVED IN CHILDREN WHO RESIDED HERE DURING THE SUSCEPTIBLE PERIOD

FIGURE 2

Table 2.—*Summary of mottled enamel findings in certain communities of eastern South Dakota with a common water supply, but where an insufficient number of examinations precluded the computation of a mottled enamel index*

Town and population (Census of 1930)	Total number of children examined	Children classified according to mottled enamel diagnosis						History of water supply	Remarks
		Normal	Questionable	Very mild	Mild	Moderate	Severe		
AGAR (200)									
(*a*) Continuous residence	23	3	0	0	2	0	0	From a 4-inch 1,800-foot well installed in 1926.	All children 4th to 8th grade, inclusive, examined.
(*b*) Nearby rural		6	0	1	0	0	0		
(*c*) Discontinuities		6	3	2	0	0	0		
BRENTFORD (174)									
(*a*) Continuous residence	42	1	1	1	1	0	0	From a 3-inch 1,200-foot well drilled in 1917.	All children 4th to 8th grade, inclusive, examined. A mottled enamel of moderate severity is being developed in immediate rural district.
(*b*) Nearby rural		1	2	3	8	6	1		
(*c*) Discontinuities		5	1	3	2	6	0		
FORESTBURG (300)									
(*a*) Continuous residence	41	5	0	0	0	0	0	From a 3-inch 740-foot well installed in 1915.	All children 4th to 8th grade and 10th grade examined. Mottled enamel being developed in immediate rural district.
(*b*) Nearby rural		10	1	0	3	0	0		
(*c*) Discontinuities		15	1	3	0	3	0		
LANE (187)									
(*a*) Town and rural (continuous).	27	20	1	6	0	0	0	From a 1¼-inch 780-foot well drilled in 1928 and continuous use since.	All children 3d to 12th grade of continuous residence examined. Mottled enamel mostly from rural district.
PUKWANA (307)									
(*a*) Continuous residence	3	2	1	0	0	0	0	From 2 900-foot wells 6-inch and 3-inch installed in 1910 and 1916, respectively. Wells were recased to 3-inch and 2-inch, respectively, in 1933.	Only 3 children 1st to 8th grade have continuously used city water. Others use individual wells or cisterns.
ROSWELL (116)									
(*a*) Continuous residence	16	3	2	0	0	0	0	From a 2-inch 611-foot well installed in 1912.	All children in school examined. One "moderate" case reports using water from a 380-foot well.
(*b*) Nearby rural		6	0	0	0	1	0		
(*c*) Discontinuities		2	2	0	0	0	0		
WHITE LAKE (530)									
(*a*) Continuous residence	24	2	0	0	0	0	0	From a 4½-inch 907-foot well drilled in 1923.	5th and 6th grades examined; town has a common water supply, but residents use mostly cistern water.
(*b*) Discontinuities		19	2	1	0	0	0		
Total	176	---	---	---	---	---	---		

TABLE 3.—*Summary of mottled enamel findings in certain communities of eastern South Dakota having no common water supply and 2 places where the common water supply is not used.*

Town and population (Census of 1930)	Total number of children examined	Children classified according to mottled enamel diagnosis						History of water supply	Remarks
		Normal	Questionable	Very mild	Mild	Moderate	Severe		
ARTESIAN (556)									
(a) Town and rural (continuous).	51	12	5	6	3	4	0	City has a 4-inch 700-foot well installed in 1905 which with a number of individual wells are connected to a common distribution system.	All children 3d to 8th grades examined.
(b) Discontinuities		13	4	1	3	0	0		
EUREKA (1,308)									
(a) Town and rural (continuous).	35	17	1	0	0	0	0	From a 3-inch 2,300-foot well installed in 1919. For domestic purposes residents use cistern and shallow well water almost exclusively.	All of 4th grade examined. In addition all of 5th and 6th grade were questioned and no child found who used city water continuously.
(b) Discontinuities		16	1	0	0	0	0		
HERREID (544)									
(a) Town (continuous)	62	25	2	0	0	0	0	No common water supply. Residents obtain domestic water from 12-15-foot dug wells and cisterns.	All children 3d to 6th grades, inclusive, examined.
(b) Rural (continuous)		5	0	0	0	0	0		
(c) Discontinuities		29	1	0	0	0	0		
VAYLAND (550)									
(a) Town and rural (continuous).	19	5	2	2	0	0	0	No common water supply.	All children 1st to 8th grades examined.
(b) Discontinuities		8	1	1	0	0	0		
VILAS (106)									
All pupils present	8	7	1	0	0	0	0	No common water supply.	Enrollment of school: 9.
WOONSOCKET (1,108)[1]									
Total	175	----	----	----	----	----	----		

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[1] Due to extreme hardness and magnesium sulfate content of city water, common water supply little used for domestic purposes. A few children in 3d and 4th grades stated they had used the common water supply but it was not possible to find a single pupil in the 5-8 grades who had used city water continuously. Local superintendent of education states there are approximately 300 individual wells in the community. Mottled enamel observed in children using water from artesian wells in this locality.

TABLE 4.—*Clinical differences following a change in the common water supply at Andover, S. Dak.*

Age at time of examination	18-14	13-11	10-7
Water used during period of tooth calcification.	Deep well (artesian)	Both supplies	Dug shallow well.
Number of children examined	13	8	14.
Percentage with mottled enamel	69	37	0.

TABLE 5.—*Analyses of selected South Dakota common water supplies* *

No.	City or town	Residue on evaporation	Loss on ignition	Fixed residue	Silica (SiO_2)	Iron (Fe)	Aluminum (Al)	Calcium (Ca)	Magnesium (Mg)	Sodium and potassium (calculated as Na)	Carbonate (CO_3)	Bicarbonate (HCO_3)	Sulphate (SO_4)	Nitrate (NO_3)	Chloride (Cl)	Phosphate (PO_4)	Boron (B)	Fluoride (F)	No.
		Parts per million (p. p. m.)																	
1	Hecla	3,000	28	2,972	12.0	0.12	0.5	54.3	5.2	965.5	0	311.1	1,407.9	5.3	375.0	0	2.4	5.0	1
2	Britton	2,568	26	2,542	23.2	.10	.3	25.7	3.5	870.4	0	341.6	1,132.0	4.4	333.0	0	2.8	6.6	2
3	Lake Preston	2,193	23	2,170	11.6	.14	.1	14.3	4.8	746.0	8.4	409.9	980.0	9.7	191.5	.1	3.5	5.9	3
4	St. Lawrence	2,134	94	2,040	13.2	.14	.2	132.2	38.4	494.5	0	192.7	1,113.2	9.7	153.0	0	1.1	2.9	4
5	Pierpont	2,459	22	2,437	12.8	.08	.6	14.3	5.2	827.6	0	453.8	1,087.6	8.8	235.5	.5	2.6	4.7	5
6	Wolsey	2,132	90	2,042	12.8	.17	.6	140.0	32.8	485.7	0	170.8	1,114.0	9.7	154.0	0	1.1	2.4	6
7	Platte	1,894	33	1,861	9.6	.18	.3	22.9	8.7	685.8	0	534.3	255.1	7.9	601.5	0	3.2	3.0	7
8	Redfield	2,260	62	2,198	22.0	.10	.5	40.0	4.4	722.7	0	261.0	1,117.4	8.8	209.0	0	2.1	2.7	8
9	Iroquois	2,116	16	2,100	15.2	.18	.3	11.4	3.5	704.6	0	301.3	1,069.6	8.4	156.0	0	1.6	2.1	9
10	Doland	2,276	21	2,255	12.8	.08	.3	17.2	.9	787.5	36.0	268.4	941.2	6.2	333.0	0	1.5	2.9	10
11	Ipswich	2,192	198	1,994	18.4	.15	.3	323.0	65.5	211.1	0	191.5	1,234.2	4.0	67.0	0	.2	2.8	11
12	Gettysburg	2,062	14	2,048	22.4	.10	.1	14.3	.9	768.0	0	744.2	355.5	8.8	499.5	.1	3.0	2.4	12
13	Miller	2,152	114	2,038	16.4	.08	.2	185.8	52.4	394.9	0	178.1	1,178.7	7.0	107.0	0	1.2	2.2	13
14	Carthage	2,143	40	2,103	10.0	.08	.2	41.4	15.3	654.6	0	235.4	1,035.9	11.0	218.5	0	1.1	2.6	14
15	Aberdeen (West Hill)[1]	2,145	148	1,997	10.4	.06	.6	240.0	53.3	325.7	0	170.8	1,222.6	14.0	67.0	0	.4	1.8	15
16	Aberdeen (Washington St.)[1]	2,183	169	2,014	16.8	.07	.6	217.2	34.1	388.6	0	175.6	1,234.2	14.4	76.0	0	.4	1.6	16
17	Leola	2,228	36	2,192	11.2	.06	.3	34.3	3.5	814.0	32.4	327.1	312.6	7.9	560.0	0	3.8	2.3	
18	Onida	1,988	72	1,913	16.0	.06	.6	111.5	9.6	522.6	0	195.2	949.4	9.7	218.5	.1	1.4	2.6	18
19	Howard	2,176	132	2,044	28.0	.08	.1	215.5	57.7	372.9	0	317.2	1,147.0	8.8	71.0	0	1.0	1.4	19
20	Andover	1,726	272	1,454	34.4	.07	.3	217.3	94.3	134.7	0	424.5	829.4	4.0	16.0	0	0	0	20

* Water samples were collected during the survey, in April and May 1938.

[1] Samples from wells of the "old" supply; a "new" impounded surface water supply was installed in 1935.

NOTE.—Assistant Chemist C. G. Remsburg carried out the determinations other than flouride and boron, using mostly the methods given in the Standard Methods of Water Analysis of the American Public Health Association. The phosphate was determined colorimetrically by an adaptation of the Benedict and Theis Method (J. Biol. Chem., 61: 63 (1924)). The boron determinations were made essentially by the method of Foote (J. Ind. Eng. Chem., Anal. Ed., 4: 39 (Jan. 15, 1932)).

PRODUCTION OF MOTTLED ENAMEL HALTED BY A CHANGE IN COMMON WATER SUPPLY

H. TRENDLEY DEAN and FREDERICK S. MCKAY *

In the United States at present there are about 375 known areas, divided among 26 states, where mottled enamel of varying degrees of severity is found. This large number of areas made available for study various communities whose common water supplies contained fluorides in different concentrations. This, in turn, permitted detailed quantitative study of concomitant variations.

A recent report (*1*) summarized studies on the quantitative relation between the fluoride concentration of the common water supply and the clinical effect. Quantitative increase in the fluoride concentration of the domestic water was concurrent with an increase in the amount and degree of clinical severity. The direct verification of concomitant variation is shown in table I. In order to present these tabular data graphically, a numerical index of clinical severity was computed by giving a definite weight to each of the several degrees of clinical affection. The following weights were given to each diagnosis: Normal, 0; Questionable, 0.5; Very Mild, 1; Mild, 2; Moderate, 3; and Severe, 4. The weighted index of clinical severity was then plotted against the fluoride concentration of the communal water supply. Figure II shows a rather precise quantitative correlation.

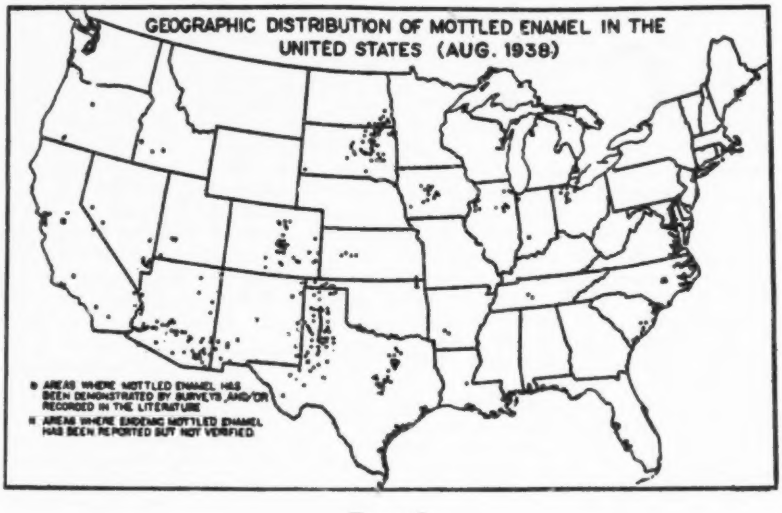
GEOGRAPHIC DISTRIBUTION OF MOTTLED ENAMEL IN THE UNITED STATES (AUG. 1938)
● AREAS WHERE MOTTLED ENAMEL HAS BEEN DEMONSTRATED BY SURVEYS AND/OR RECORDED IN THE LITERATURE
■ AREAS WHERE ENDEMIC MOTTLED ENAMEL HAS BEEN REPORTED BUT NOT VERIFIED

FIGURE I

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* Read before the Epidemiology Section of the American Public Health Association at the Sixty-seventh Annual Meeting in Kansas City, Mo., October 25, 1938.

CONSEQUENCE OF CHANGE IN THE COMMON WATER SUPPLY

A more cogent type of epidemiological evidence is now added to that shown by concomitant variations. Three communities where the endemicity of mottled enamel had been confirmed by survey, changed their communal water supply from one containing a fluoride concentration now known to be sufficient to produce endemic mottled enamel to one practically free of fluorides. Each community presented the indispensable conditions of a susceptible population using the "new" water supply and a sufficient lapse in time (8–10 years) for a study of the consequent differences. The two sets of circumstances were similar in all respects save for the one difference, the change in the water supply. The surveys made prior to the change in the water supply and the resurvey made after the requisite lapse of time were made by either one or the other of the writers. The communities studied were: Oakley, Ida., Bauxite, Ark., and Andover, S.D.

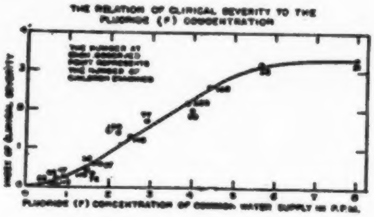


FIGURE II

Oakley, Ida.—The first instance of a community changing its public water supply solely for the purpose of preventing mottled enamel is that of Oakley, Ida. A survey of this community was made by one of us (2) in February, 1925.

Seventy-eight children whose histories indicated continuous residence and uninterrupted use of the common water supply were examined. The incidence of affection was 100 percent. The common water supply of Oakley at the time of the survey was obtained from a warm spring. Following the survey, the community on July 1, 1925, changed its water supply, the new supply being obtained from a nearby cold water spring. The selection of this water was based on the observation that the 4 children of a family using this spring were free of mottled enamel.

Approximately 7½ years (Feb. 1933) after the change in the common water supply, Oakley was resurveyed (*3*). Twenty-four children born since the change in the water supply were examined. *All showed normal calcification* in those permanent teeth which at that time had erupted. According to analyses (*3*) made by Churchill, the "old" Oakley water contained 6.0 p.p.m. of fluorine; the "new" supply, less than 0.5 p.p.m.

TABLE I.—*Illustration of Quantitative Relation Between the Fluoride (F) Concentration of the Common Water Supply and Severity of Clinical Affection*

City and State	*Number of Children Examined*	*Mean Annual Fluoride (F) Content in p.p.m.*	*Percentage Incidence of Affection*	*Percentage Distribution of Clinical Severity* — *Pathognomonic Signs* — *Absent* — *Normal*	*Quest.*	*Present* — *Very Mild*	*Mild*	*Moderate*	*Severe*	*Age Group or School Grade Examined*
Puebio, Colo.	83	0.6	2.4	88.0	9.6	2.4	...	...	...	9 Year
Jct. City, Kans.	115	0.7	1.7	95.7	2.6	1.7	...	...	...	9–10–11 yrs.
Big Spring, Tex.	68	0.7	3.0	85.3	11.7	3.0	...	...	...	5th, 6th Gr.
Mullins, S. C.	47	0.9	10.6	68.1	21.3	8.5	2.1	...	...	9–10–11 yrs.
East Moline, Ill.	110	1.5*	24.5	55.5	20.0	18.2	5.4	0.9	...	"
Webster City, Iowa	72	1.6	26.4	65.3	8.3	22.2	4.2	...	...	"
Monmouth, Ill.	38	1.7	42.1	36.8	21.0	36.8	5.3	...	...	9 Year
Galesburg, Ill.	57	1.8	35.1	45.6	19.3	26.3	5.3	3.5	...	"
Clovis, N. M.	138	2.2	71.0	13.0	16.0	23.9	35.4	11.0	0.7	9–10–11 yrs.
Colo. Spr., Colo	148	2.5	67.6	18.2	14.2	28.4	21.6	14.2	3.4	9 Year
Plainview, Tex.	77	2.9	87.0	3.9	9.1	33.7	24.7	24.7	3.9	9–10–11 yrs.
Amarillo, Tex.	229	3.9†	89.5	3.5	7.0	16.6	24.9	34.5	13.5	"
Conway, S. C.	59	4.0	88.2	5.1	6.7	20.4	32.2	23.7	11.9	"
Lubbock, Tex.	168	4.4	97.5	1.2	1.2	10.7	24.4	44.0	18.5	"
Post, Tex.	38	5.7	100.0	...	...	....	10.5	50.0	39.5	4, 5, 6 Gr.
Ankeny, Iowa	21	8.0‡	100.0	...	...	....	9.5	47.6	42.8	2–12 Gr.

* Subject to possible correction to 1.3 p.p.m.
† Subject to possible correction to 4.2 p.p.m. during susceptible period of the age group examined.
‡ Single determination, all others, arithmetical mean of 12 consecutive monthly samples.

(Computed largely from data recorded in *Public Health Reports*, 52:1249–1264 (Sept. 10), 1937, and *American Journal of Public Health*, June, 1936.)

Bauxite, Ark.—In February 1928, a mottled enamel survey of Bauxite was made by Kempf and McKay (*4*). Sixty of the 62 children of continuous residence examined showed mottled enamel, generally of a severe type. Of the two classified as normal, Kempf and McKay recorded in a footnote that although the homes of these children were piped for the city water, it appears that it was only used occasionally for domestic purposes. The water used by the 60 affected children examined during the 1928 survey was obtained from deep wells and, according to Churchill (*5*), contained 13.7 p.p.m. of fluoride (F).

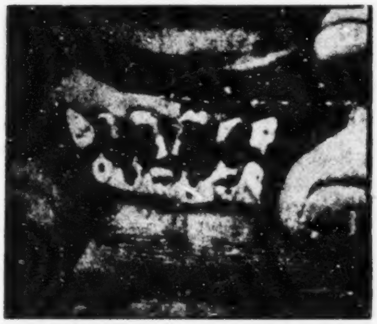
In May 1928, the Bauxite water supply was changed, the "new" supply being obtained from the nearby Saline River. A resurvey (*6*) by the writers in March, 1938, showed that of the 45 children born since the change in the water supply, only 2 showed even the mildest form of mottled enamel. A possible explanation of the two "very mild" cases is contained in the detailed report of the Bauxite resurvey (*Pub. Health Rep.*, 53: 1736–1748 (Sept. 30), 1938). The fluoride (F) content of the "old" Bauxite water was so unusually high, 13.7 p.p.m., that the question of antepartum maternal fluorosis and subsequent transfer of small amounts of fluorides in the mother's milk cannot be excluded. The 7-year-old child with the "very mild" diagnosis was nursed 15 months. Consideration might also be given to possible fluoride deposits from the "old" supply in the water pipes and cooking utensils. The "new" filtered river water supply is practically free of fluorides, containing less than that determinable routinely (0.2 p.p.m.).

Andover, S.D.--In 1916, one of us (F. S. McK.) made a qualitative survey of the school children of Andover and found mottled enamel endemic, associated with the use of the water from the city artesian well supply (*7*). In 1928 this deep well water supply failed and the community was forced to change from the 800-foot artesian to a dug shallow well 22 feet deep. The fluoride content of the "old" Andover (S.D.) deep well water is not known, but presumptive evidence based on clinical mottled enamel among those who calcified their permanent teeth while using this water indicates that it contained fluorides, probably in the neighborhood of 2.5–3.00 p.p.m. of fluorine. Wells of comparable depth in the immediate rural district are likewise producing mottled enamel. An examination of the Andover school children by one of the writers (H.T.D.) in April 1938, showed that the 14 children, 6–10 years of age, born since the change in the water supply, presented normal calcification of the permanent teeth. The "new" water supply used by these children was found to be free of toxic amounts of fluoride.

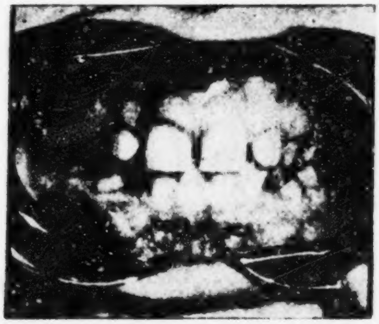
DISCUSSION

An analysis of the findings, by age groupings, of the resurveys recorded in table II shows the striking differences resulting from the mere change in the water supply. Those in the higher age groups who calcified their permanent teeth while using the "old" water supply

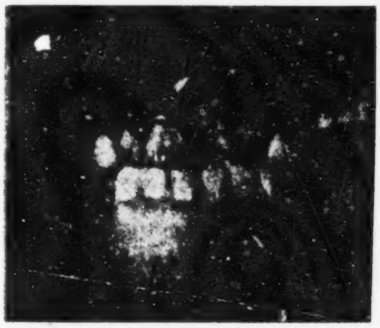
Children Who Calcified Their Permanent Teeth While Using:



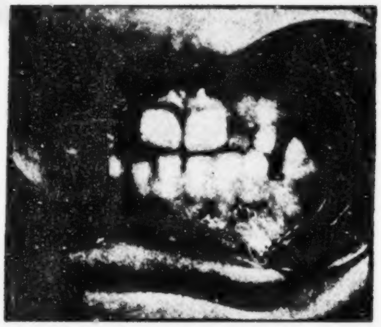
"Old" Bauxite Water
Mottled Enamel: Severe



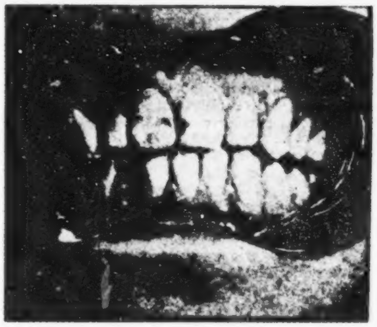
"New" Bauxite Water
Normal



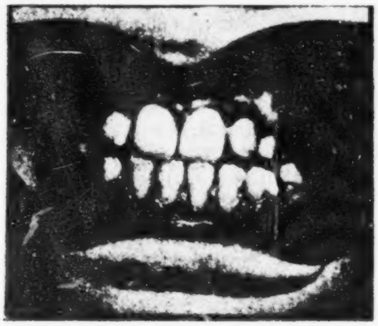
"Old" Oakley Water
Mottled Enamel: Moderate



"New" Bauxite Water
Normal



"Old" Oakley Water
Mottled Enamel: Mild



"New" Oakley Water
Normal

show an incidence of mottled enamel comparable to that observed during the original survey. Next a definite decrease in the percentage incidence of affection becomes manifest in those children whose dental calcification period overlapped the change in the water supply (age group who calcified their teeth while using both water supplies), and finally the relative freedom from mottled enamel in those born subsequent to the change in the water supply. These decreasing percentage incidences of clinical affection speak for themselves. With respect to Oakley, they were 92, 20, and 0; Bauxite, 100, 52, and 4; and Andover, 69, 37, and 0, respectively.

While we are fully aware that the number of observations is comparatively small, the effects noted are consistent. The observations, moreover, were limited to those children constantly exposed to the risk of the disease, namely, they were born in the community, had in general always resided there, and had always used the common water supply for both drinking and cooking. In the case of the resurvey at Bauxite, the facts with respect to residence and water consumption were verified in each instance by an interview with the child's parent.

In specific water-borne diseases, the time intervening between the elimination of the antecedent cause and the non-appearance of the pathological entity is relatively short. The opposite characteristic of endemic dental fluorosis (mottled enamel) makes it unique among the water-borne diseases. An interval in time between 8 to 10 years is required to carry out a human experiment that furnishes clinical proof that the development of the disease has stopped.

With the report on the results obtained at Oakley, Ida., Bauxite, Ark., and Andover, S.D., showing that the production of mottled enamel has actually been stopped by the mere change in the water supply from one containing concentration of fluorides toxic to calcifying dental enamel to one practically free of fluoride (or less than 1.0 p.p.m.), the most conclusive and direct proof that fluoride in the drinking water is the primary cause of human mottled enamel has been presented. The separate steps in the complete chain of evidence may now be summarized as follows:

1. The evidence pointing to mottled enamel being a water borne disease (*8, 7*).
2. The finding of fluorides in domestic waters associated with the production of mottled enamel (*9, 5, 10*).
3. The experimental production of dental lesions in white rats and dogs by waters from endemic areas and waters to which fluoride has been added (*9, 11, 12*).
4. Quantitative epidemiological studies on human mottled enamel correlating the fluoride concentration of the domestic water with the degrees of clinical severity (*13, 1*).
5. And finally, after the lapse of the necessary interval of 8 to 10 years, the clinical evidence that the production of human mottled enamel had been halted by merely changing the common water supply from one containing fluorides in concentrations toxic to calcifying dental enamel to one practically free of fluorides (*3, 6*).

SUMMARY

1. The production of the endemic hypoplasia of the permanent teeth known as mottled enamel has been halted at Oakley, Ida.; Bauxite, Ark.; and Andover, S.D., by simply changing the common water supply from one containing amounts of fluorides toxic to calcifying dental enamel to one whose fluoride content does not exceed the permissible maximum, 1 p.p.m.

2. Oakley, Ida., and Bauxite, Ark., are the first known instances of communities abandoning an otherwise satisfactory common water supply solely for the

Table II.—*Summary of the findings in surveys of three communities where the production of mottled enamel was halted by a change in the common water supply*

		Over 15	15	14	13	12	11	10	9	8	7	6	Incidence of mottled enamel among users of "old" water supply	Fluoride (F) content of "old" water supply	Incidence of mottled enamel among users of "new" water supply	Fluoride (F) content of "new" water supply
FINDINGS OF ORIGINAL SURVEY																
AGE OF CHILD AT TIME OF EXAMINATION		Over 15	15	14	13	12	11	10	9	8	7	6				
OAKLEY, IDAHO FEB. 1925	WATER USED-CALCIFICATION PERIOD	WARM SPRING SUPPLY														
	NUMBER OF CHILDREN EXAMINED	78														
	% HAVING MOTTLED ENAMEL	100														
BAUXITE ARKANSAS FEB. 1928	WATER USED-CALCIFICATION PERIOD	DEEP WELL WATER														
	NUMBER OF CHILDREN EXAMINED	3	1	3	7	2	2	4	8	10	13	9				
	% HAVING MOTTLED ENAMEL	66	100	100	100	100	100	100	100	100	100	89				
		96 *														
ANDOVER SO. DAK. OCT. 1916	WATER USED-CALCIFICATION PERIOD	DEEP ARTESIAN WELL														
	NUMBER OF CHILDREN EXAMINED	NOT STATED														
	% HAVING MOTTLED ENAMEL	QUALITATIVE SURVEY ONLY MEDIUM DEGREE OF ENDEMICITY														
FINDINGS OF RESURVEY													%	P.P.M.	%	P.P.M.
AGE OF CHILD AT TIME OF EXAMINATION		OVER 15	15	14	13	12	11	10	9	8	7	6				
OAKLEY IDAHO FEB. 1933	WATER USED-CALCIFICATION PERIOD	WARM SPR. SUPPLY						BOTH SUPPLIES			COLD SPR.		92	6.0	0	<0.5
	NUMBER OF CHILDREN EXAMINED	-	15			10	3	11	15	14	16	8				
	% HAVING MOTTLED ENAMEL	-	93			100	66	36	15	14	0	0				
		92						20			0					
BAUXITE ARKANSAS MAR. 1938	WATER USED-CALCIFICATION PERIOD		DEEP WELL		BOTH SUPPLIES			FILTERED RIVER WATER					100	13.7	4	0.
	NUMBER OF CHILDREN EXAMINED	-	7	7	10	6	7	5	13	8	14	5				
	% HAVING MOTTLED ENAMEL	-	100	100	80	50	14	0	8	0	7	0				
			100		52			4								
ANDOVER SO. DAK. APR. 1938	WATER USED-CALCIFICATION PERIOD	DEEP ARTESIAN WELL			BOTH SUPPLIES			SHALLOW DUG WELL					72	†	0	0
	NUMBER OF CHILDREN EXAMINED	6	5	2	2	5	1	6	2	0	6	0				
	% HAVING MOTTLED ENAMEL	85	60	50	50	40	0	0	0	-	0	-				
		67			37			0								

* IN THE TWO NORMAL CASES AUTHORS RECORD IN FOOT NOTE, DISCONTINUITIES IN USE OF COMMON WATER SUPPLY.

† ON THE BASIS OF CLINICAL EVIDENCE, ARTESIAN WELL SUPPLY PRESUMABLY CONTAINED

definite purpose of preventing mottled enamel; and in itself likewise represents the first instance where a common water supply was changed solely for the purpose of preventing a dental disease.

3. The unusually long interval in time (8–10 years) before the clinical effects resultant from a change in the water supply may be observed clinically, is unique in epidemiological investigations of water-borne diseases.

4. With the report on the results obtained in three endemic areas, showing that the production of mottled enamel has actually been stopped by the mere change in the water supply from one containing concentrations of fluoride toxic to calcifying dental enamel to one practically free of fluoride, the most conclusive and direct proof that fluoride in the domestic water is the primary cause of human mottled enamel, has been presented.

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(*11*) Sebrell, W. H., Dean, H. T., Elvove, E., and Breaux, R. P. Changes in the Teeth of White Rats Given Water from a Mottled Enamel Area Compared with Those Produced by Water Containing Sodium Fuoride. Pub. Health Rep., *48*: 437–445 (Apr. 28), 1933.

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CLIMATE AND ENDEMIC DENTAL FLUOROSIS

By Donald J. Galagan and Glenn G. Lamson

Investigations demonstrating the relationship between trace amounts of fluoride in community water supplies, the prevalence of mottled enamel, and a reduction in dental caries experience have been extensive during the last half century. By 1931 it had been determined that mottled enamel was associated with the presence of fluoride in drinking water (*1, 2*). Subsequent studies revealed the direct quantitative relationship between fluoride and mottled enamel (*3–6*) and demonstrated the inverse relationship between fluoride and dental caries experience (*7*).

Based upon these data, using the community fluorosis index to measure the extent of mottled enamel (*8*), and the decayed, missing, filled (DMF) index as a measurement of caries experience (*9*), an optimum fluoride concentration was derived for use in the supplementation of fluoride-deficient community water supplies. The range of fluoride concentration most effective in preventing dental caries was established at approximately 1.0 to 1.5 p.p.m., well below the critical point in the causation of mottled enamel.

A review of the literature reveals that the recommended fluoride levels have been determined from observations made within a fairly limited geographic area, principally in the Midwest (*10*) where mean annual temperatures are approximately 50° F.

Temperatures in the continental United States vary widely, from an average mean annual temperature of 40° to 45° F. in the northern tier of States to 70° F. and above in some areas of California, Arizona, Texas, Louisiana, and Florida (fig. 1).

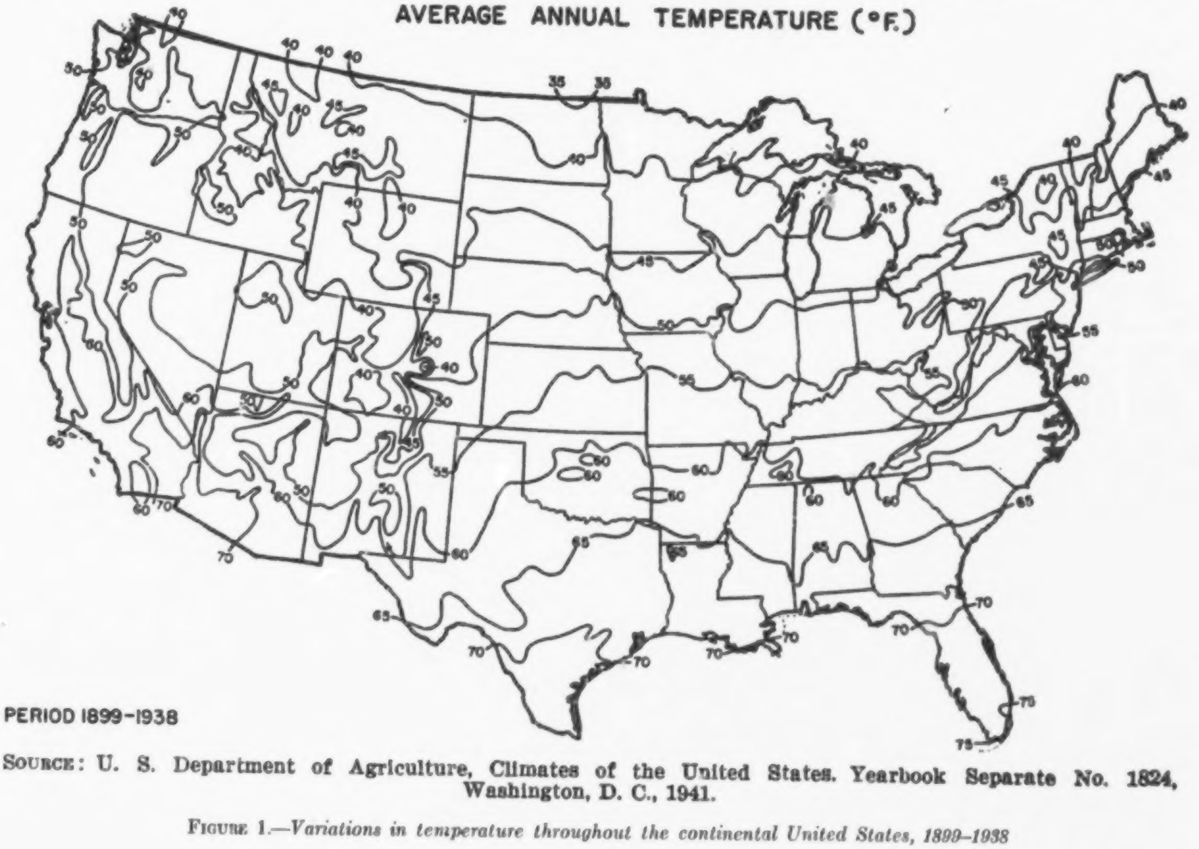
In view of the temperature distribution in the United States, a fluoride concentration of 1.0 to 1.5 p.p.m. seems justifiable for the major portion of the country. However, Arnold (*11*) has suggested that because of climatic conditions 1.0 to 1.5 p.p.m. of fluoride may not be practical or desirable in every community, and Dean has recently indicated that less than 1.0 p.p.m. of fluoride may represent the optimal level for dental caries control in a southern community (*12*). The early findings of the Smiths (*13, 14*), while not relating mottled enamel to climatic factors, certainly suggest that mild fluorosis is associated with less than 1.0 p.p.m. of fluoride in certain areas in Arizona.

Recognition of a probable relationship between fluoride concentration and climate is being observed in the present fluoridation effort. For example in the State of Wisconsin, 1.3 p.p.m. is recommended. In Charlotte, N.C., the concentration of fluoride is varied from a high of 1.2 p.p.m. in winter to a low of 0.6 p.p.m. in summer, and in Florida, 0.7 p.p.m. is the recommended optimum amount. Nevertheless, if climatologic factors markedly influence the water intake of infants and youth, additional information about optimum concentrations should be obtained for those exceptional areas experiencing extreme climatic conditions.

Since the severity of mottled enamel has a specific relationship to the fluoride content of water consumed, the application of a simple biological test such as a measurement of fluorosis may be used to evaluate water intake indirectly. In order to use this method of assessing climatic forces upon water intake, it seemed logical to measure fluorosis in communities with extreme temperatures. If objectionable fluorosis was not associated with fluoride levels around 1.0 p.p.m. in the areas with extremely high temperatures, then that concentration would be desirable in the supplementation of fluoride-deficient water in areas with similar climate, and at least that amount would be required in all other parts of the United States.

FIELD STUDIES

The Arizona communities of Yuma, Tempe, Tucson, Chandler, Casa Grande, and Florence were selected for initial study. With the exception of Yuma these communities lie south and east of Phoenix on the plains of the Arizona Desert. Yuma is situated on the Yuma Desert on the northern edge of the Great Desert of Sonora, Mexico.



SOURCE: U. S. Department of Agriculture, Climates of the United States. Yearbook Separate No. 1824, Washington, D. C., 1941.

FIGURE 1.—*Variations in temperature throughout the continental United States, 1899–1938*

These six communities met the necessary requirements for investigation. They had water supplies with adequate continuity and with fluoride concentrations ranging around 1.0 p.p.m. They were of sufficient size to yield a sample of native-born children large enough to be significant (*15*). They had mean annual temperatures ranging from 67° to 72° F., with an average mean annual temperature of approximately 70° F. This area is consistently reported as one of the hottest inhabited areas in the United States, exceeded in temperatures only by certain communities adjacent to Death Valley, Calif.

Public Water Supplies

The general characteristics of the water systems of the six study communities are shown in table 1. The data on fluoride determinations of the common water supplies of the six communities are presented in table 2.

Table 1.—*Characteristics of the common water supplies in six Arizona communities, 1935-51*

Community	Source of supply	Treatment
Yuma	Colorado River.	Desilting; aluminum sulfate; flocculation; copper sulfate; filtration; chlorination.
Tempe	4 wells	Chlorination.
Tucson	17 wells	Chlorination; ammoniation.
Chandler	4 wells	None.
Casa Grande	5 wells	Do.
Florence	4 wells	Do.

These data were available as samples taken from individual wells and from the general distribution system after pooling. The arithmetical mean of all available analyses of individual well water and of finished water has been accepted as the figure most nearly representing the fluoride concentration of the several water supplies. They represent analyses made intermittently from 1935 through 1950 and quarterly during 1951. The fluoride concentrations range from 0.4 p.p.m. at Yuma to 1.2 p.p.m. at Florence.

A more detailed description of each of the water supplies follows.

Yuma. The public water supply of Yuma has been obtained from the Colorado River since 1892. The treatment plant in current use was put into operation in 1945. The first treatment starts about 18 miles above Yuma, where a desilting plant is located. The main treatment plant is situated on the Arizona bank of the Colorado River in Yuma proper. A 4,500-gallons-per-minute turbine pumps water directly into the first settling basin in the plant. A second turbine, with a 5,000-gallons-per-minute capacity, pumps water from the Bureau of Reclamation canal, 1,200 feet away. Treatment at the plant consists of the addition of aluminum and copper sulfate, flocculation, filtration, and chlorination. Storage is obtained by means of two 500,000-gallon reservoirs from which the water is pumped into the distribution system.

(In the summer of 1937, sewage backed up in the Colorado River, and the water became unsafe for drinking purposes. During a 3-month period water from the City Park well, normally used to supply the municipal swimming pool, was pumped into the distribution system and supplied the water for the community. No data are available indicating the fluoride concentration of the well water at the time it was used for drinking purposes, but a sample of the water as of May 12, 1951 showed 0.6 p.p.m.)

The mean of 79 fluoride determinations shows the Yuma water supply to have a fluoride concentration of 0.4 p.p.m.

Tempe. During the study period the water for the community of Tempe was obtained from four wells. These wells, established in 1894, were located side by side, were pumped by a single piston, and were approximately 140 feet deep. No data are available to show whether they were cased, but the local waterworks operator thought they probably were not.

Finished water is stored in a 1,000,000-gallon tank. Chlorination was instituted in 1939; no other treatment is given. The average fluoride concentration for the common water supply is 0.5 p.p.m.

Tucson. The city of Tucson presented a special investigative problem. The water supply for the community is derived from more than one source, and is distributed through two systems, the Northside system with a fluoride concentration of about 0.3 p.p.m. and the Southside system with a fluoride concentration of 0.7 p.p.m. This study was concerned exclusively with the area served by the Southside system and hereafter will be the one under consideration.

The water for the Southside system is obtained from ground water in the Santa Cruz River basin. It is produced from 17 wells located south of the city on the east bank of the river (dry). The water from these wells is pumped into a 30-inch concrete conduit which carries it to two reservoirs, with 1,500,000- and 7,300,000-gallon capacities, respectively. The water is chlorinated at a point in the conduit before arriving at the reservoir. This is followed by ammoniation at 0.05 p.p.m.

Fourteen of the 17 wells were in operation prior to 1930. Two additional wells were placed in use in 1946, one in 1947. The wells are pumped alternately in the winter; in the summer, when demand is greater, all wells operate most of the time. The first 6 wells sunk are 125 feet deep. The remaining 11 extend from 200 to 300 feet in depth. Their individual output varies from 300 to 1,300 gallons per minute, with a total capacity of 9,000 gallons per minute.

Table 2.—*Reported fluoride concentration of water from all available single source and distribution system samples in six Arizona communities, 1935-51*

Community	Number of samples	Mean fluoride content
Yuma	79	0.4
Tempe	7	.5
Tucson	31	.7
Chandler	16	.8
Casa Grande	20	1.0
Florence	22	1.2

Sources: University of Arizona, College of Agriculture, Agricultural Experiment Station, Tucson, Ariz.; U.S. Department of Agriculture, Bureau of Plant Industry, Soils and Agricultural Engineering, Salinity Laboratory, Riverside, Calif.

Since 1938, when a set of control valves was installed to separate the two systems, no water has passed from the Northside into the Southside system. Prior to 1938, small amounts of water may have passed from the Northside into the Southside system. Since the fluoride concentration of the Northside system is the lesser of the two, the contamination of the water under investigation, if any, would result in a lowered fluoride concentration.

Only children who had continuous exposure to the common water supply of this community within a community were included in the study group. A buffer zone of three to five blocks was set up within the limits of the Southside system. Children were excluded from the study if they had lived in or beyond the buffer zone at any time. The normal range of a child at play probably is not greater than that; consequently, exposure to fluoride concentrations less than that of the Southside system would be casual.

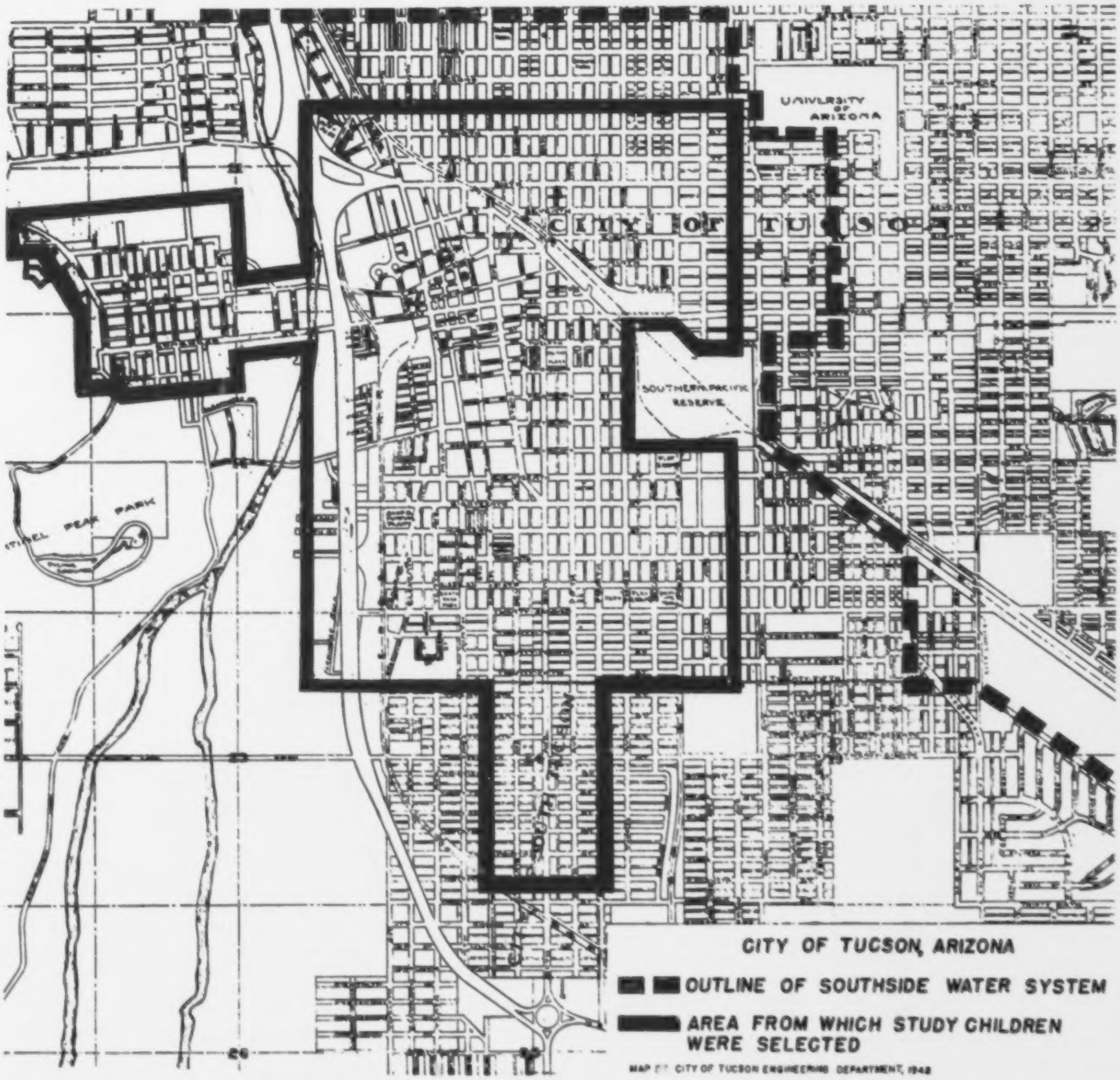
The broken line on the map of the city of Tucson (fig. 2) shows the limits of the Southside water system as of 1935. The solid line indicates the area from which the study children were selected.

Chandler. During the study period the water supply for the city of Chandler has been produced by four wells. The initial two wells, 335 and 987 feet deep, were drilled and put into operation in December 1926. Both wells drew water from the 300- to 325-foot level, the depth at which they were perforated. In 1938 the casing above the perforations broke, and a considerably harder water began to enter the wells. Because of this, two new wells were put into operation, one in 1944 and one in 1948. They are both 650 feet deep, perforated from the 360-foot level to the bottom. The original wells were sealed off in September 1944.

The arithmetical mean of the fluoride determinations made while the original wells were in use is 0.75 p.p.m.; of those made subsequent to that date, 0.85 p.p.m. The single analysis available for the period during which water was entering the wells from above the perforations indicates that the fluoride concentration at that time was 0.8 p.p.m. the same as the numerical average for all observations. It seems clear that the fluoride concentration of the common water supply has remained constant during the study period.

Casa Grande. The water for Casa Grande is obtained from five wells, which were put into operation in 1922, 1930, 1937, 1946, and 1950. The location of each new well site was moved consistently to the northeast to get better water, but they are in fairly close proximity, all but one being within the city limits. The wells range in depth from 210 to 302 feet, with the exception of one 750-foot well which is used for emergency purposes only. No data were available as to the depth of the perforations in the well casing.

The wells have a total rated output


UNIVERSITY OF ARIZONA
CITY OF TUCSON
SOUTHERN PACIFIC RESERVE
SENTINEL PEAK PARK
SOUTH TUCSON
CITY OF TUCSON, ARIZONA
▬ ▬ OUTLINE OF SOUTHSIDE WATER SYSTEM
▬ AREA FROM WHICH STUDY CHILDREN WERE SELECTED

MAP BY CITY OF TUCSON ENGINEERING DEPARTMENT, 1948

FIGURE 2.—*Map of the city of Tucson, Ariz., showing outline of Southside water distribution system, buffer zone, and area from which study children were selected.*

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of 1,700 gallons per minute with an average reported daily output of 594,000 gallons. The water is stored in a ground level storage tank of 500,000-gallon capacity and in an elevated tank holding 100,000 gallons. It is not treated.

The new wells added to the system during the present study do not seem to have changed the fluoride concentration of the water supply. Samples from the distribution system have ranged from 0.9 p.p.m. to 1.2 p.p.m. of fluoride. Twenty analyses, for the years 1931 through 1951, are available, representing all seasons of the year. The numerical average of all available fluoride concentrations reported for samples taken from the system and from individual wells is 1.0 p.p.m.

Florence. During the study period the community of Florence has obtained its water from four wells. The two original wells, 180 and 190 feet deep, were put into operation in 1919. A new 400-foot well, perforated the last 40 feet, was put into operation in 1939, and the two old wells were closed. Because of increased demand, another 400-foot well, perforated at the same level, was put into operation in July 1947. The new and the original wells are adjacent to each other within the city limits.

The wells now in use have a rated output of 350 gallons per minute, and an average daily output of 386.5 thousand gallons per day. The water is not treated. Overflow is stored in a 55,000-gallon elevated standpipe.

Since there was a complete change in well usage during the early part of the study period, the available fluoride concentrations have again been divided into those taken prior to the date the old wells were closed, July 1939, and those taken after that date. A total of 21 fluoride analyses, with a numerical average of 1.2 p.p.m., was reported during the study period. Fourteen of the samples were taken prior to July 1939, and 7 were taken after that date. Both average 1.2 p.p.m. of fluoride. It seems evident that the fluoride content of the Florence water supply was not altered by the change in wells.

Materials and Methods

Since these studies were designed to determine the extent of dental fluorosis in children with continuous exposure to common water supplies containing low fluoride concentrations, it was necessary to get accurate information regarding the water history of the children studied. The following screening procedure was adopted in each community:

Screening Step 1. Public and parochial school children from the fourth through the ninth grades were each issued a card which asked the following questions:

Table 3.—*Percentage age distribution of 726 children examined in six Arizona communities, 1951*

Number of children in each community	Age (years) and percent in each age group							
	9	10	11	12	13	14	15	16
Yuma (82)	5	9	7	10	7	18	16	28
Tempe (113)	5	13	16	18	11	12	13	12
Tucson (316)	3	7	8	11	18	24	17	12
Chandler (95)	8	15	14	20	15	9	12	7
Casa Grande (50)	8	12	22	12	18	14	8	6
Florence (70)	3	9	9	16	18	14	20	11

Were you born in this town?

Have you lived here all your life?

Have you been away from this town for more than 30 days in any 1 year?

This initial screening served to eliminate those children obviously not continuous residents of the community.

Screening Step 2. Each child who indicated that he was born in the community and had lived there all his life was given a questionnaire to take home to his parents or guardian, with an explanatory letter. The parents were requested to record the birthplace of the child, the addresses of all his residences, continuously and in sequence, from birthdate to date of the questionnaire, and the source of the water used for drinking and cooking purposes at each residence. They were also asked to describe specifically the absences of the child from the community for more than 30 days.

Screening Step 3. Children who passed through the first two steps were scheduled for dental examination. At the time of examination, each child was questioned by a dentist or a dental hygienist to verify the information obtained in steps 1 and 2. A school or public health nurse was present to add her knowledge of the home situation to the water history data.

Screening Step 4. If the data obtained in any of the first three steps was inconsistent the parent was interviewed by a member of the survey team and was asked to clarify the discrepancies.

Only children who had consumed water from the common municipal supply continuously from birth through their ninth year were included in the study. Children who experienced interruption in the continuity of consumption of the community water for periods of more than 30 days in any one calendar year were excluded. The remaining children served as study material.

Clinical Examinations

All dental examinations were made by the same examiner. The children were seated in a portable dental chair; and a mouth mirror, a Burton light, and compressed air were available for the examiner's use. Each tooth was assigned a fluorosis classification according to Dean (*8*). The tooth-unit fluorosis classification was later transposed into child-unit classification for use in computing the community fluorosis index. The percentage age distribution of the 726 Arizona children examined is shown in table 3.

Findings

The data relative to the prevalence and severity of fluorosis obtained in the six communities are presented in table 4. The occasional child falling into the moderate or severe classification when exposed to relatively low fluoride concentrations is worthy of note.

The community fluorosis index may be used for an objective measurement of the exent of endemic dental fluorosis. The direct relationship between fluoride concentration and fluorosis noted in prior investigations is evident. As the fluoride concentration rises the community fluorosis index is increased and the number of children without visible fluorosis is reduced. The community fluorosis index ranges from 0.21, associated with 0.4 p.p.m. of fluoride at Yuma, to 1.12 for Florence which has 1.2 p.p.m. of fluoride in its water supply.

COMPARISON WITH MIDWESTERN INDEXES

To compare possible variations in the intensity of dental fluorosis under different climatological conditions, data obtained from 10 communities with similar fluoride concentrations were selected from the study of "21 cities" by Dean and his coworkers (*10*). The communities in the 21 cities group, which may be considered as a base line, have a mean annual temperature of approximately 50° F., whereas the six communities in Arizona have a mean annual temperature of approximately 70° F.

Data on the prevalence and severity of fluorosis in both groups of communities are presented in table 5.

Comparison of the data reveals that the Arizona communities have a somewhat higher percentage of children affected by fluorosis than communities with a cooler climate and comparable fluoride concentrations in their water supplies. There is also a wider distribution throughout the fluorosis classification. Some of the Arizona children present moderate to severe fluorosis associated with exposure to less than

TABLE 4.—*Prevalence of fluorosis, distribution of signs of fluorosis and community fluorosis indexes in relation to fluoride concentrations of common water supplies continuously used by 726 children examined in six Arizona communities, 1951*

Community	Fluoride concentration	Number children examined	Number children affected	Number of examined children with signs of fluorosis						Community fluorosis index
				Fluorosis absent		Fluorosis present				
				Normal	Questionable	Very mild	Mild	Moderate	Severe	
Yuma	0. 4	82	3	53	26	2	1	--------	--------	0. 21
Tempe	. 5	113	11	59	43	10	1	--------	--------	. 30
Tucson	. 7	316	53	120	143	38	10	5	--------	. 46
Chandler	. 8	95	18	40	37	9	6	2	1	. 52
Casa Grande	1. 0	50	24	7	19	15	9	--------	--------	. 85
Florence	1. 2	70	39	17	14	18	10	9	2	1. 12

TABLE 5.—*Prevalence of fluorosis, percentage distribution of signs of fluorosis and community fluorosis indexes in relation to fluoride concentration of common water supplies of 16 communities in two temperature zones*

Community	Fluoride concentration	Community fluorosis index	Number children examined	Percent children affected	Percentage distribution of signs of fluorosis						Mean annual temperature ° F.[1]
					Absent		Present				
					Normal	Questionable	Very mild	Mild	Moderate	Severe	
Arizona											
Yuma	0. 4	0. 21	82	4	65	32	2	1	--------	--------	72. 2
Tempe	. 5	. 30	113	10	52	38	9	1	--------	--------	68. 6
Tuscon	. 7	. 46	316	17	38	45	12	3	2	--------	67. 4
Chandler	. 8	. 52	95	19	42	39	9	6	2	1	67. 6
Case Grande	1. 0	. 85	50	48	14	38	30	18	--------	--------	71. 0
Florence	1. 2	1. 12	70	56	24	20	26	14	13	3	69. 3
Midwest											
Marion, Ohio	. 4	. 25	263	6	57	37	5	1	--------	--------	52. 1
Elgin, Ill	. 5	. 22	403	4	61	35	4	1	--------	--------	49. 4
Pueblo, Colo	. 6	. 17	614	7	72	21	6	(²)	--------	--------	52. 6
Kewanee, Ill	. 9	. 31	123	12	53	35	10	2	--------	--------	50. 9
Aurora, Ill	1. 2	. 32	633	15	53	32	14	1	--------	--------	49. 4
East Moline, Ill	1. 2	. 49	152	32	37	32	30	2	--------	--------	50. 9
Maywood, Ill	1. 2	. 51	171	33	39	28	29	4	--------	--------	50. 1
Joliet, Ill	1. 3	. 46	447	25	41	34	22	3	--------	--------	49. 4
Elmhurst, Ill	1. 8	. 67	170	40	28	32	30	9	1	--------	50. 1
Galesburg, Ill	1. 9	. 69	273	48	25	27	40	6	1	--------	50. 9

[1] Average: Arizona, 69.3° F.; Middle West, 50.6° F.

[2] Less than 0.5 percent.

NOTE: Age range for Arizona group, 9–16 years; midwestern group, 12–14 years.

SOURCES: Fluorosis data for midwestern communities from Dean, H. T.: Epidemiological studies in the United States. American Association for the Advancement of Science: Dental caries and fluorine, Science Press, Lancaster, Pa., 1946; mean annual temperature for 6 Arizona communities from Smith, H. V.: The climate of Arizona. University of Arizona, Agricultural Experiment Station, Bulletin No. 197, July 1945; for midwestern communities; from U. S. Department of Commerce, Weather Bureau: Climatological data. Monthly and annual summaries: The mean annual temperature for Aurora, Joliet, and Elgin is represented by the 19-year average mean temperature for Aurora; that for Kewanee, East Moline, and Galesburg by the 19-year average mean temperature for Galva; and that for Maywood and Elmhurst by the 19-year average mean temperature for Chicago.

the generally recommended fluoride concentration of 1.0 p.p.m.

Dean has stated that a community fluorosis index below 0.4 is of little or no public health concern. He considers the range from 0.4 to 0.6 as borderline. For indexes above 0.6 the removal of excessive fluorides from the water is recommended (*8*). From table 5 it may be seen that the fluorosis indexes for communities with fluoride concentrations above 0.5 p.p.m. are considerably higher in the Arizona communities. Yuma, Ariz., and Marion, Ohio, both have fluoride concentrations of 0.4 p.p.m. and fluorosis indexes of 0.21 and 0.25, respectively. As the fluoride concentration rises, the disparity between indexes becomes marked, so that Florence, Ariz., and East Moline, Ill., with fluoride concentrations of 1.2 p.p.m., present fluorosis indexes of 1.12 and 0.49, respectively.

The fluorosis indexes for the two groups of communities have been plotted on figure 3.

The least squares method was used to calculate the index lines. (Trend line formula for 70° F. communities is $y = -0.291 + 1.132x$ and for 50° F. communities is $y = 0.021 + 0.353x$.) The line for the Arizona group has a much steeper slope than that representing the midwestern communities. The index line for the Arizona communities accelerates at approximately twice the rate as the one for the midwestern communities. The line for the midwestern cities crosses from the negative area into the borderline zone at approximately 1.1 p.p.m. and from the borderline into the objectionable zone at 1.7 p.p.m. On the other hand, the line for the Arizona communities crosses from the negative area into the borderline zone at 0.6 p.p.m. and into the objectionable zone at 0.8 p.p.m.

These data would indicate that the children residing in the Arizona communities under consideration develop twice as much fluorosis as midwestern children when exposed to water containing the same concentration of fluoride. (It should be noted that the two groups of children were diagnosed by different examiners. The data are therefore subject to the error of examiner differences.)

CLIMATOLOGICAL VARIABLES

About two-thirds of man's total fluid intake is water, the remainder, other fluid substances. Except for temporary circumstances of an emotional nature, the amount of fluid ingested is determined by water deficiency. Every bit of body water lost must be replaced, and the replacement amounts are obligatory. (In growing children fluid intake may be slightly greater than water loss since some additional water is needed to build new tissues, but generally speaking, water intake and water loss will be equal over a 24-hour period.)

TEMPERATURE

Excessive temperatures result in a bodily demand for fluid over and above that usually required for normal physiological processes (*16*). When environmental temperatures rise above skin temperature (92° to 95° F.), the only method by which the body can cool itself is vaporization. Heat loss can no longer be effected by radiation or conduction. The water output of the body is therefore increased in proportion to the need for increased vaporization. It follows that there will be an equal increase in the amount of water ingested in order to maintain body water balance.

The extremely high temperature occurring in the southwestern communities is undoubtedly a major factor contributing to the increased severity of endemic fluorosis observed in the Arizona children through its influence on water consumption. Some indication of the difference in temperature between the two geographic areas is reflected in their mean annual temperatures of 50° and 70° F. (table 6).

TEMPERATURE RANGE

The mean annual temperature is a simple measure of the climatic conditions to which man is exposed. It is the mean of the daily high and low temperatures for a year, and does not critically reflect difference in daytime and nighttime temperatures.

During most of their tooth-forming years children are active almost exclusively during daytime hours. Consideration of mean temperature alone may therefore result in an underestimation of the actual temperatures to which they have been exposed in warm, semi-arid climates. Comparisons of temperature ranges may reflect more clearly the real difference in temperature experienced by the children residing in two geographic locations. The data on mean maximum and mean minimum temperatures and the temperature ranges for the Arizona communities under consideration, and for the midwestern communities (or representative communities if complete weather data for each city were not available) are also presented in table 6.

Although the difference between the mean annual temperatures of the two groups of communities is considerable, the difference in the range of high and low temperatures, or day and night temperatures, is even more marked. The midwestern communities have an average range of 22° F. between the daytime maximum and the nighttime minimum, whereas the Arizona communities have an average range of 33° F. Daily variations are considerably greater in any semiarid area (*17*), and daytime temperatures well above 100° F. are common for many weeks in the Arizona communities. The interpretation of these data makes even more striking the difference in temperatures to which the children of the two zones are exposed.

TABLE 6.—*Annual mean maximum, mean minimum, mean temperature, and temperature range for 6 Arizona communities and 5 representative*[1] *midwest communities*

Community (station)	Number of years of record	Temperature (° F.)			
		Mean maximum	Mean minimum	Mean	Range
Arizona					
Yuma Citrus Station	20	87.2	56.9	72.2	30.3
Tempe No. 2	15	84.3	53.0	68.6	31.3
Tucson (University of Arizona)	49	82.5	51.3	67.4	31.2
Chandler	21	85.3	50.3	67.6	35.0
Casa Grande	26	87.8	51.7	71.0	36.1
Florence	26	86.3	52.7	69.3	33.6
Midwest					
Marion, Ohio	19	62.4	42.3	52.1	20.1
Pueblo, Colo	19	67.3	36.8	52.6	30.5
Aurora, Ill	19	60.5	39.0	49.4	21.5
Chicago, Ill	19	58.4	42.7	50.1	15.8
Galva, Ill	19	62.2	40.6	50.9	21.6

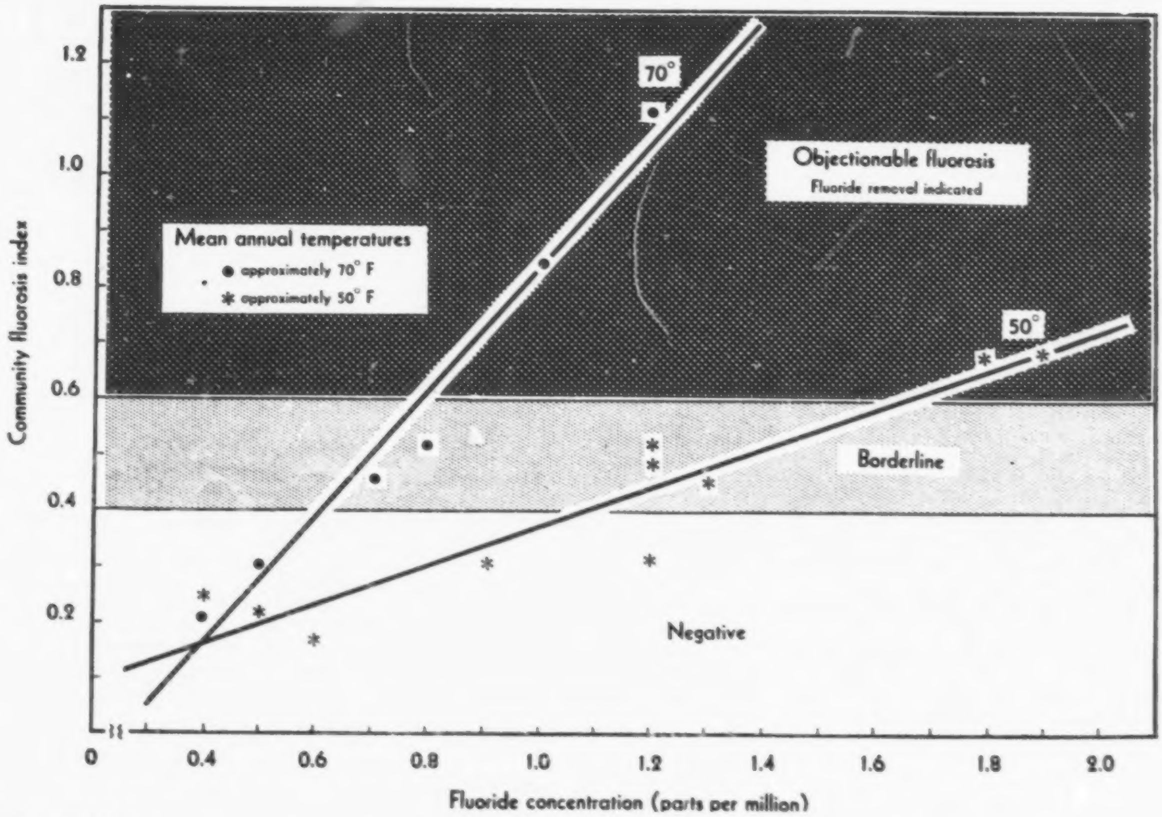
[1] Aurora represents Aurora, Joliet, and Elgin; Chicago represents Maywood and Elmhurst; Galva represents Kewanee, East Moline, and Galesburg.

SOURCES: For Arizona communities: Smith, H. V.: The Climate of Arizona. University of Arizona, Agricultural Experiment Station, Bulletin No. 197, July 1945; for midwestern communities: U. S. Department of Commerce, Weather Bureau, Climatological data, monthly and annual summaries.

RADIANT HEAT GAIN

Water needs vary with other factors beside temperature. Body weight, physical activity, direct radiation, and humidity may all play a part in the amount of fluid lost by the body and therefore in the amount of fluid ingested to maintain water balance. Certain of these variables, such as body weight and physical activity, which contribute to physiological heat gain, will obviously not differ greatly between geographic areas. Other factors contributing to environmental heat gain should be taken into consideration, however.

Individuals exposed to the same air temperatures and different amounts of direct sunlight are subject to varying influences on water loss. It has been demonstrated that there is an increase in the sweating rate of about 20 grams per hour for each degree of increase in air



Source: Table 5.

FIGURE 3.—*Relationship between fluoride concentration of municipal waters and fluorosis index for communities with mean annual temperatures of approximately 50° F. (Midwest) and 70° F. (Arizona).*

temperature. Direct sunlight causes an elevation in the sweating rate equivalent to that resulting from a 10° F. increase in air temperatures (*18*). Therefore, exposure to direct sunlight increases water loss by increasing radiant heat gain. An indication of this factor may be obtained from a determination of the amount of sunshine in an area.

The part of Arizona under consideration has a greater percentage of possible sunshine than any other area in the United States—80 to 85 percent compared to 50 to 60 percent in the Chicago area (*17*). Therefore in the southwest desert there is extremely high radiant heat gain from the sun. This climatic factor, causing a degree of environmental heat stress not reflected in temperature measurements alone, may also indirectly account for some of the observed regional differences in endemic fluorosis.

RELATIVE HUMIDITY

The effect of humidity upon water loss is not as clear as that of temperature and radiation. Studies by Adolph (*18*), Rubner (*19*), and Benedict and Carpenter (*20*) suggest that a lower relative humidity tends to increase water loss, temperatures being equal. On the other hand, Newburgh (*21*) points out that since moisture demand is decreased in humid air, more skin surface has to be wetted (and more sweat produced) to achieve the equivalent of cooling experienced in dry, absorbent air of comparable temperature. Therefore, a humid atmosphere would tend to increase water loss merely by making the body secrete more sweat in order to gain the same amount of evaporative cooling accomplished in arid areas with less water loss. Since the data appear to be conflicting, the influence of relative humidity upon the water intake of infants and children is difficult to assess.

Considerable difference does exist between the relative humidities of the two areas in the study. For example, the annual mean relative humidity for the Tucson-Yuma area is 35 to 37 percent, and for the Chicago area, 74 percent (*17*). The data available indicate that the Arizona communities have relative humidities approximately half that observed in the midwestern communities. On hot summer afternoons the relative humidity in the Arizona desert may be 5 percent or less.

It is questionable whether the marked difference in relative humidity between the two areas is influential in the water intake of infants and children. Measurement of its influence is further complicated by the difficulty encountered in obtaining comparable humidity data and by the fact that other meteorological conditions appear to have a greater effect on water loss.

NONCLIMATIC VARIABLES

The Arizona area under consideration is desert country with very little rainfall. Consequently, all productive land is under irrigation. Since many water supplies in Arizona contain fluoride in some amount, the soil may potentially have fluoride added to it by irrigation. Smith and associates (*22*) were unable to show any appreciable increase in the uptake of fluoride by grains and vegetables, even when concentrations up to 3,200 p.p.m. were artificially added to the soil in which they were grown. In a comprehensive review of the literature, McClure (*23*) concludes that fluoride in soil has little or no influence on the fluoride content of edible plant produce. Increased fluoride intake through use of food products grown in soil irrigated with water containing the amounts of fluoride naturally occurring in Arizona therefore seems extremely unlikely.

One other nonclimatic factor should be recognized when attempting to account for the observed regional differences in the severity of fluorosis. Generally speaking, the children included in the Arizona group were of Spanish descent (83 percent). Their dietary staple, beans, is usually prepared by boiling for rather long periods of time. Since boiling in fluoride-bearing water results in a concentration of the fluoride ion in many cooked vegetables (*24*), there may have been an increase in the dietary fluoride intake of this group to a greater degree than would be observed for a group of children on a more varied diet. It was not within the scope of this study to measure the influences of dietary variables upon fluoride intake.

SUMMARY

1. The prevalence and severity of endemic dental fluorosis in 726 children were studied in six Arizona communities. The cities were located in a desert area, with mean annual temperatures of approximately 70° F., mean relative humidities of approximately 37 percent, 80 to 85 percent of possible sunshine, and extremely high daytime temperatures.

2. In water supplies of the Arizona communities studied, concentrations of fluoride above 0.8 p.p.m. resulted in objectionable dental fluorosis; concentrations of 0.6 to 0.8 p.p.m. resulted in an occasional diagnosis of fluorosis; concentrations below 0.6 p.p.m. did not cause objectionable fluorosis.

3. Comparisons of the community fluorosis indexes for the Arizona communities with those obtained from selected midwestern communities indicate that fluorosis occurs at about twice the intensity in that section of Arizona as it does in the midwestern area with comparable fluoride concentrations but markedly different climatic factors.

4. Variation in temperature, radiant heat gain and relative humidity have been discussed as possible influential factors in the observed differences in endemic fluorosis between the two areas.

5. The application of a simple biological test in two areas suggests that, because of several climatological influences, Arizona children drink more water than children living in more temperate climates. As a result, there is increased ingestion of fluoride in relation to the concentration found in the water supply.

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The information concerning the Arizona water supplies was obtained from George W. Marx, director, bureau of sanitation, Arizona State Department of Health, and from the local waterworks superintendent in each community.

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THE RELATION OF DENTAL CARIES TO FLUORIDE DRINKING WATERS

Children whose drinking water has contained continuously a source of fluoride sufficient to produce mottled enamel have also acquired a significant immunity to dental caries. This widely observed association lead to the hypothesis that this particular caries immunity was due to water-borne fluoride and could be operative without producing mottled enamel. Major epidemiological studies verified this hypothesis conclusively. They demonstrated that drinking waters containing 1.00 p.p.m. of fluoride as a natural constituent are significantly caries preventive and do not produce mottled enamel.

The partial control of dental caries through the direct addition of fluoride to a community water supply thus became a truly momentous possibility. It gave rise to major problems involving preventive dentistry, human fluoride physiology and community public health.

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ENDEMIC FLUOROSIS AND ITS RELATION TO DENTAL CARIES

By H. TRENDLEY DEAN

INTRODUCTION

The first thorough study of mottled enamel, that of Black (*1*) and McKay (*2*) at Colorado Springs and including the Pike's Peak watershed, reported as early as 1916 that, in regard to caries, the teeth of these Colorado children compare favorably with those of other communities where endemic mottled enamel is unknown. Black also wrote of the difficulty of successfully filling carious mottled enamel teeth and stated that, though the percentage of carious teeth is less than in nonendemic areas, probably a greater proportion of filled teeth are eventually lost because of the difficulty of retaining fillings in the hypoplastic tooth structure.

Workers in other countries have also commented on the qualitative aspects of this phenomenon. Mottled enamel is endemic in the southwestern part of the Japanese Archipelago. Masaki (*3*) reported 18 endemic areas in the Prefectures of Hyogo, Fukuoka, Ehime, Hiroshima, and Aichi, 12 of the 18 being located in Hyogo and Fukuoka. In an English abstract of his original report, this investigator states that "It is also remarkable that the percentage of dental caries is comparatively small among those who suffer this abnormality." The number of observations upon which this generalization was based is not stated in the abstract.

Ainsworth has commented on the lessened prevalence of dental caries among children in the endemic areas of Maldon and Heybridge, Essex County, England. In connection with the studies of the Committee for the Investigation of Dental Diseases, of the Medical Research Council, this investigator (*4*) examined approximately 4,000 children in the public elementary schools in various parts of England and Wales. He states (*5*) that the condition of the teeth in the Council schools at Maldon and Heybridge was generally good, being well above the average for Council schools. He specifically notes that "there was relatively little caries: 7.9 percent of the permanent teeth were carious, as compared with an average in all districts examined of 13.1 percent; and 12.9 percent of deciduous teeth were carious against 43.3 percent in all districts." The percentages just quoted are reported as corrected for age distribution (*4*) in the different schools.

Erausquin (*6*) who has studied mottled enamel extensively in the Argentine Republic records that there appears to be an inverse variation between dental caries and "dientes veteados," the name by which endemic dental fluorosis is known in the Argentine. He stated, however, that the findings were not conclusive on the basis of the limited number of areas studied.

Probably the first attempt to study specifically the relationship of mottled enamel to dental caries was made by McKay (*7*) who, in 1929, attacked the hypothesis that dental decay might be superinduced by "defective" enamel structure, by citing as evidence the observation that mottled enamel teeth, which probably constitute "the most poorly constructed enamel of which there is any record in the literature of dentistry," do not appear to show any greater liability to dental caries than do normally calcified teeth.

His report refers to studies made at Bauxite (Ark.), Minonk (Ill.), Towner (Colo.), Bruneau (Idaho), and the Pima Indian School at Sacaton (Ariz.). Certain tabulated data from the last three named places are included in McKay's report. Table 1 has been compiled from certain of these data.

In 1933–34 a study was begun by the United States Public Health Service to determine the minimal threshold of toxicity of chronic endemic dental fluorosis. In this study (*8, 9, 10*) consecutive monthly water samples were received from each of the cities surveyed, which permitted the computation of an arithmetic mean annual fluoride (F) content of the communal water supply. The clinical examinations in these cities were limited to those children who were born in the community, had always resided there, and had continuously used the common water supply for both drinking and cooking.

In certain of these cities, in addition to recording the degree of severity of mottled enamel, each child was examined for other defects of the enamel, such as present caries, past caries (fillings or extractions), pits and fissures, hypoplasias, etc. The examinations were made in a good light with the child seated facing a window. Mouth mirrors free from blemishes and new explorers were used. For each child examined in connection with the caries aspects of the study, the facts with respect to residence and continual use of the common water supply were verified by an interview with the child's parent or guardian.

The amount of caries recorded may appear somewhat higher than usual; for, in addition to definite cavitation, defects in the enamel on caries-susceptible surfaces showing either a discoloration or opacity around the edges and in which an explorer would cling, were counted as caries. All examinations were made, however, by one individual, the writer.

An analysis of these data indicates that a higher percentage of caries-free children is found in cities whose water supplies contain relatively toxic amounts of fluorides than in those communities with water supplies not so affected. Since in certain cities only the 9 year old children were examined, comparisons will be limited to children of this age. It was decided also to omit cities where less than 25 children were examined at this age. Table 2 presents the pertinent data.

The data shown in table 2 indicate a

TABLE 1.—*Variation in prevalence of dental caries in normal and mottled enamel teeth of three endemic areas according to McKay*

Locality	Number of children examined	Total number of permanent teeth examined	Number of teeth examined and percentage with dental caries				
				All teeth		Molar teeth	
				Number examined	Percent carious	Number examined	Percent carious
Towner, Colo. (Pop. 154 in 1930).	[1] 55	1,264	Normal teeth	879	11	254	36
			Mottled enamel teeth	385	9	101	33
Bruneau, Idaho (Pop. 481 in 1930).	[1] 54	1,142	Normal teeth	356	16	126	46
			Mottled enamel teeth	797	8	213	29
Pima Indian School, Sacaton, Ariz. (Pop. unstated).	[1] 78	2,178	Normal teeth	283	22	99	57
			Mottled enamel teeth	1,895	14	529	45

[1] Age, sex, color, continuity of residence, and constancy of exposure to the mottled enamel-producing waters not recorded in the report.

Table 2.—*Percentages of caries-free children, 9 years of age, in 6 selected cities classified according to their continuous use of water of different fluoride (F) concentration*

Locality	Actual community mottled enamel index	Domestic water supply[1]		Number of children examined
		Fluoride (F) content	Total hardness	
		p. p. m.	*p. p. m.*	
Pueblo, Colo	Negative	0.6	303	49
Junction City, Kans	Negative	0.7	277	30
East Moline, Ill	Border-line	[2] 1.5	242	35
Monmouth, Ill	Slight	1.7	288	29
Galesburg, Ill	Slight	1.8	237	39
Colorado Springs, Colo	Slight	2.5	20	54

[1] For detailed mineral analyses of these waters, see ref. *(8)* *(10)*.
[2] Subject to possible correction to 1.3 p. p. m.

Table 2.—*Percentages of caries-free children, 9 years of age, in 6 selected cities classified according to their continuous use of water of different fluoride (F) concentration*—Continued

Locality	Caries-free children					
	All teeth		Permanent		Deciduous	
	Number	Percent	Number	Percent	Number	Percent
Pueblo, Colo	3	6	18	37	4	9
Junction City, Kans	0	0	8	26	1	3
East Moline, Ill	2	6	4	11	8	23
Monmouth, Ill	6	21	16	55	6	21
Galesburg, Ill	8	20	22	56	11	28
Colorado Springs, Colo	13	24	22	41	21	40

Fluoride content in p. p. m.	Number examined	Composite sample of above 9-year-old children classified on the basis of exposure to domestic water of lower and higher fluoride (F) concentration					
0.6–1.5	[3] 114	5	4	30	26	13	11
1.7–2.5	[4] 122	27	22	60	49	38	31

[3] Of this group, 51 were boys and 63 girls; by color, 108 white and 6 colored.
[4] In this group 59 were boys and 63 girls; classified according to color, 116 white, 4 colored, and 2 Mexican.

greater freedom from dental caries in the 122 children exposed to domestic waters of higher fluoride (F) concentration, both with respect to permanent and deciduous teeth. It is a well known fact that deciduous teeth are seldom affected with mottled enamel; in this particular group, only three children, all of Colorado Springs, showed even the mildest forms of mottled enamel in their deciduous teeth, generally in the second deciduous molars. Of the 122 children in the group, 60 were caries-free with respect to the permanent teeth. Of these 60, 33, or 55 percent, were affected with mottled enamel. In the whole group (122), the incidence of mottled enamel was 53 percent. These observations suggest that the limited-immunity-producing factor present in the water is operative whether or not the tooth is affected by mottled enamel. Whether this mechanism functions locally, systematically, or both ways, is not known.

Relation of Endemic Fluorosis to Dental Caries in Large Population Groups

Source of data.—The disclosure of an inverse relation between the prevalence of dental caries and the fluoride concentration of the domestic water supply, as shown in table 2, raises the question of the kind of relationship between these two variables in other and larger population groups. The requisite data on dental caries are provided by the dental survey of school children 6–14 years of age, made in 26 States in 1933–34 *(11)* under the direction of the United States Public Health Service. This survey included a total of 34,283 examinations of white children in South Dakota, 15,465 in Colorado, and 48,628 in Wisconsin, made by dentists reported as using a mouth mirror and explorer in making the examinations. Furthermore, these examinations were made on a standard examination form and largely for the purpose of recording the amount of dental caries in the school population; the marked differences, therefore, in the amount of caries noted in groups using domestic waters of different mineral composition takes on an added significance.

Data on mottled enamel, on the other hand, are furnished by a recent (1938) survey of South Dakota made by the writer. During this survey (April–May 1938), approximately 3,300 school children in 51 communities were examined for mottled enamel, and endemic mottled enamel was demonstrated in 35 communities, each having a common water supply. A comparable degree of mottled enamel was widely prevalent in the surrounding rural districts in certain of the counties, ascribable to the general custom of farmers of obtaining their domestic water supply from artesian wells in the Dakota sandstone. Moreover, the examination of school children with discontinuities in their residence pointed to 21 other places in the State, not as yet surveyed, but where, on the basis of clinical signs present in the children, mottled enamel is endemic.

Method of analysis.—All South Dakota counties listed in Bulletin No. 226 *(11)* in which 35 percent or more of the estimate population of ages 6–14 years had been examined, were selected for study. On the basis of the mottled enamel data, these counties were divided into three groups: (*a*) Counties where mottled enamel is prevalent, (*b*) counties where mottled enamel distribution is uneven, and (*c*) counties which, so far as we know, are entirely free from mottled enamel. Both the 1933–34 dental needs survey and the 1938 mottled enamel studies were made in those South Dakota counties lying east of the Missouri River.

In computing an index which might point out differences in dental caries in the several counties, it was decided to express the amount of caries (severity) in terms of the number of carious permanent teeth per 100 children. In order to study that age group with the maximum number of permanent teeth in the mouth, the 12–14 year group was selected for study. All children referred to in the tables to follow are white. The amount of caries was determined by combining the data associated with the following items: "Caries, permanent teeth," "Extraction indicated, permanent teeth," "Filled permanent teeth," and "Extracted permanent teeth." For each of these items, the bulletin gives the number of carious permanent teeth per 100 children. Adjustment was made for sex, and the amount of caries for each county was expressed in terms of the number of carious permanent teeth per 100 children. In Public Health Bulletin No. 226, examinations from communities with a population under 5,000 were combined with examinations from the rural areas and designated "balance of county."

The South Dakota counties selected from the bulletin were classified solely on the basis of the prevalence of or freedom from endemic mottled enamel as shown by the mottled enamel study. The cities of Aberdeen, Huron, and Sioux Falls were classified on the basis of whether or not the common water supply was producing mottled enamel. The results of these computations are contained in table 3. Similar computations were also made for four Colorado cities and eight Wisconsin cities; the results are shown in table 4.

DISCUSSION

This paper, after reviewing the findings of the earlier workers in the field, submits evidence that furnishes support to the hypothesis that a limited immunity from dental caries is operative among school children residing in endemic mottled enamel areas. This evidence may be summarized as follows:

Prevalence.—Observations made on a selected sample of 9-year-old children continuously exposed to waters of different fluoride concentrations, with the history of exposure personally verified in each instance by an interview with the child's parent, indicate that a high percentage of children are caries-free in those places where the common water supply contains appreciable amounts of fluorides. For instance, of the 114 children who had continuously used a domestic water comparatively low in fluorides (0.6 to 1.5 p.p.m.), only 5, or 4 percent, were caries-free. On the other hand, of the 122 children of comparable age who had continuously used domestic water containing 1.7 to 2.5 p.p.m. of fluorides, 27, or 22 percent, were caries-free. In other words, within the range of these observations, limited to a total of 236 9-year-old children, the percentage of caries-free children in areas with domestic water containing appreciable amounts of fluorides was over 5 times the corresponding percentage in areas with domestic water containing lower fluoride concentrations.

Severity, or amount of caries.—To provide additional evidence of the relation of dental caries to endemic fluorosis, a computation of the dental caries attack rate on the permanent teeth of 12–14-year-old children was made with the use of data collected in 1933–34 (Public Health Bulletin No. 226) and correlated with the data subsequently obtained on the geographical distribution of mottled enamel in South Dakota. Briefly, this study shows that, in the group of counties where mottled enamel is generally prevalent, an examination of 1,902 white children, 12–14 years of age, disclosed 201 carious permanent teeth per 100 children. In the intermediate group of seven counties where the mottled enamel distribution was uneven, and at times sporadic, the examination of 2,765 children showed 314 permanent teeth affected per 100 children; and in the third group of counties and the cities of Huron and Sioux Falls, where no endemic mottled enamel areas are known to exist, an examination of 3,481 children showed a dental caries attack rate of 415 permanent teeth per 100 children. These data indicate that the dental caries attack rate in this particular population is inversely proportional to the prevalence of mottled enamel.

Similar comparisons made among four cities of Colorado show that in the nonendemic communities, Pueblo, Fort Collins, and Denver, the dental caries attack rate is 194, 296, and 343, respectively; on the other hand, in the endemic area, Colorado Springs, only 163 permanent

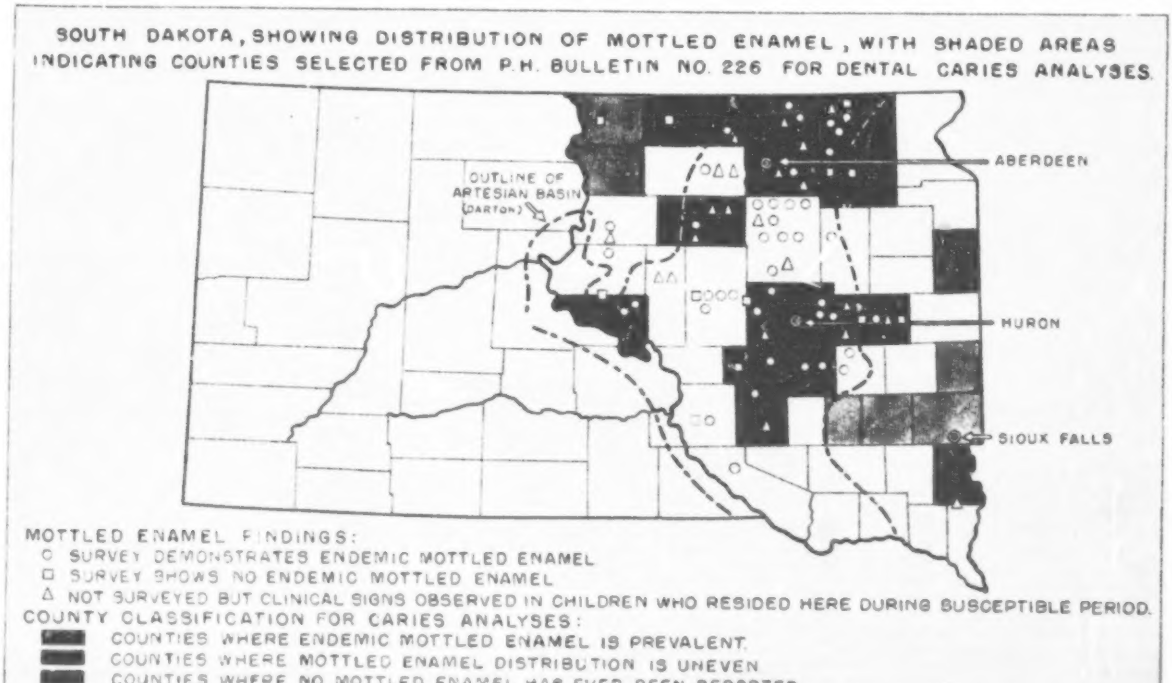
SOUTH DAKOTA, SHOWING DISTRIBUTION OF MOTTLED ENAMEL, WITH SHADED AREAS INDICATING COUNTIES SELECTED FROM P.H. BULLETIN NO. 226 FOR DENTAL CARIES ANALYSES.
OUTLINE OF ARTESIAN BASIN (DARTON)
ABERDEEN
HURON
SIOUX FALLS
MOTTLED ENAMEL FINDINGS:
○ SURVEY DEMONSTRATES ENDEMIC MOTTLED ENAMEL
□ SURVEY SHOWS NO ENDEMIC MOTTLED ENAMEL
△ NOT SURVEYED BUT CLINICAL SIGNS OBSERVED IN CHILDREN WHO RESIDED HERE DURING SUSCEPTIBLE PERIOD.
COUNTY CLASSIFICATION FOR CARIES ANALYSES:
■ COUNTIES WHERE ENDEMIC MOTTLED ENAMEL IS PREVALENT.
■ COUNTIES WHERE MOTTLED ENAMEL DISTRIBUTION IS UNEVEN
■ COUNTIES WHERE NO MOTTLED ENAMEL HAS EVER BEEN REPORTED.
□ COUNTIES WITH NO DATA OR INSUFFICIENT DATA ON PREVALENCE OF CARIES
18

FIGURE 1

teeth per 100 children were affected. Applying the same methods of study to eight Wisconsin cities, it was found that in the seven where no endemic mottled enamel is known to exist and where the fluoride (F) content of the communal water supplies ranges from 0.1 to 0.5 p.p.m., the severity of dental caries showed rates from 646 to 917 carious permanent teeth per 100 children. But in the city of Green Bay, where the city water contains 2.3 p.p.m. of fluoride (F), only 275 carious permanent teeth per 100 children were recorded.

It is fully realized, of course, that the causes of dental caries are, as Rosenau states, "complex and perhaps multiple." Mill's recent analysis of Public Health Bulletin No. 226, moreover, indicates that the dental caries attack rates (amount of dental caries) vary markedly in different geographical regions.

An inspection of the data included in this paper brings out the fact that, regardless of the dental caries attack rate of the region, the use of a domestic water which in itself is capable of producing mottled enamel is concomitant with a lower amount of dental caries.

Relation to dental caries research.—The relationship of chronic endemic dental fluorosis (mottled enamel), a water-borne disease, to dental caries raises several questions, for example:

1. What role, if any, does the physical structure of a tooth play in either susceptibility to or immunity from dental caries?

2. Is the higher fluoride content of the enamel of a mottled-enamel tooth the immunity-producing factor?

3. Is the limited immunity due, directly or indirectly, to the well-known inhibitory action of fluorine on enzymatic processes?

4. While on the basis of our present knowledge it appears justifiable to associate the observed results with the presence of fluorides in the domestic water, the possibility should not be overlooked that other elements of comparatively rare occurrence in water or ordinary constituents of drinking water present in unusually large concentration may directly or through a synergistic action with the fluoride, produce the observed effects. For this reason, it appears essential to obtain as complete chemical analyses as possible of the domestic water of communities which are under investigation for dental caries.

SUMMARY

1. Examinations of 236 9-year-old children with verified continuity of exposure showed that a higher percentage of children is caries-free in those communities where the domestic water supplies contain higher concentrations of fluorides

TABLE 3.—*Dental caries attack rates in permanent teeth of 12–14 year old white children in selected South Dakota counties and cities classified according to the prevalence of mottled enamel*

County	Number of children examined (12-14 years)	Number of carious permanent teeth per 100 children	Remarks
(a) COUNTIES WHERE MOTTLED ENAMEL GENERALLY IS PREVALENT [*]			
Beadle (less Huron)	332	256	Mottled enamel general throughout county. Areas include Hitchcock, Wolsey, Virgil, Yale, Cavour, and rural districts.
Brown (city, Aberdeen)	653	203	On basis of clinical examinations, old city deep well water contained fluorides in excess of minimal threshold.
Faulk	266	149	Mottled enamel general throughout county. Areas include Faulkton, Orient, Cresbard, Chelsea, and rural districts.
Marshall	391	251	Mottled enamel severe in western half of county, including Kidder, Britton, Langford, Newark, Amherst, and rural districts. No information on eastern half of county.
Sanborn	260	[1] 103	Mottled enamel prevalent in county including Artesian and numerous rural districts.
Total	1,902	201	
(b) COUNTIES WHERE MOTTLED ENAMEL DISTRIBUTION IS UNEVEN			
Jerauld	295	294	Alpena and Wessington Springs are negative; some mottled enamel in and around Lane.
Aurora	340	227	Mottled enamel around Stickney and rural districts in northern part of county.
Kingsbury	398	330	Distribution varied. Iroquois, Bancroft, Esmond, and Lake Preston are endemic. DeSmet and Arlington, two largest communities in county are negative.
Day	666	369	Some mottled enamel in extreme western part of county around Pierpont. Bristol and Andover are negative by survey. No indications of mottled enamel in any other section of county.
Hughes	184	206	Blunt negative for mottled enamel; cases being developed in rural district around Harrold.
McPherson	346	394	Some mottled enamel in extreme eastern part of county around Leola. Eureka surveyed and negative. County generally free of mottled enamel.
Lincoln	536	284	Some mottled enamel observed from Beresford; no other record of mottled enamel in county.
Total	2,765	314	
(c) COUNTIES WHERE NO MOTTLED ENAMEL HAS EVER BEEN REPORTED			
Beadle (city, Huron)	436	398	Negative for mottled enamel; obtains city water from James River with deep well as a reserve.
Campbell	264	368	No record of mottled enamel in this county. Herreid negative by survey.
Deuel	212	218	No reports of mottled enamel in this county.
Hanson	271	382	Do.
McCook	344	407	Do.
Minnehaha:			
City, Sioux Falls	608	451	No reports of mottled enamel in this city; State chemist reports 0.4 p. p. m. F in treated city water.
Balance of county	584	476	No reports of mottled enamel in this county.
Moody	433	498	Do.
Walworth	329	355	Do.
Total	3,481	415	

[1] Only "caries and extractions indicated" of the permanent teeth are listed in Bulletin No. 226 for this county; adjusted for sex shows 68.6 per 100 children. This figure was raised to 103.1 per 100 children in order to compensate for the "unknown" filled and extracted permanent teeth per 100 children. The increase was based on the average ratio that these two missing items bear to the "caries and extraction indicated" reported in the 4 counties adjoining Sanborn County. The percentages that these items bear to the whole were as follows: Jerauld, 31; Kingsbury, 34; Beadle (less Huron) 34, and Aurora, 35.

[*] The city of Aberdeen is included in this group of counties as this city was using a deep well water supply at the time of the dental caries survey. This water supply produced a mild degree of mottled enamel in about 20 percent of the children continuously using the water.

Table 4.—*Dental caries attack rates in permanent teeth of 12–14 year old white children of ALL Colorado and Wisconsin cities listed in Public Health Bulletin No. 226*

City	Number of children examined (12–14 years)	Number of carious permanent teeth per 100 children	Fluoride (F) content of common water supply (p. p. m.)	Reference
	Colorado			
Colorado Springs	203	162	2.5	(8)
Pueblo	411	[1] 194	0.6	(8)
Denver	637	342	0.5	(12)
Fort Collins	207	296	None	(12)
	Wisconsin			
Green Bay	687	275	[2] 2.3	....
Sheboygan	244	710	0.5	(13)
Manitowoc	661	682	0.35	(13)
Two Rivers	382	646	0.3	(13)
Milwaukee	2,645	917	0.3	(13)
West Allis	160	831	0.3	(13)
Baraboo	119	733	0.2	(13)
La Crosse	47	731	0.12	(13)

[1] "Extraction indicated" for boys "Unknown"; 4.2 rate for girls used in this adjustment.—Author.

[2] Determination made by Senior Chemist E. Elvove, Division of Chemistry, National Institute of Health. Approximately the same amount has been reported by DeWitt and Nichols (J. Am. Water Works Assoc., **29**:980-984 (July 1937).

Note.—For the mineral constituents, other than fluorine, of these Wisconsin waters, see Public Water Supplies of Wisconsin, Wisconsin State Board of Health, July 1935.

(F) in comparison with communities using waters of lower fluoride concentrations. This limited immunity to dental caries seemed operative with respect to the deciduous teeth as well as the permanent teeth.

2. An analysis of dental caries attack rates in a relatively large number of children in the three States thus far studied (South Dakota, Colorado, and Wisconsin) indicates that the severity of dental caries is, in general, lower in mottled enamel areas as compared with normal areas in the same State.

3. Inasmuch as it appears that the mineral composition of the drinking water may have an important bearing on the incidence of dental caries in a community, the possibility of partially controlling dental caries through the domestic water supply warrants thorough epidemiological-chemical study.

ACKNOWLEDGMENT

The outline of the artesian basin in eastern South Dakota shown in figure 1 was taken from plate LXIX, by N. H. Darton, in part 2 of the Seventeenth Annual Report of the United States Geological Survey, 1895–96.

The writer desires to express his indebtedness to Senior Statistician Wm. M. Gafafer and Senior Chemist E. Elvove, National Institutes of Health, for many helpful suggestions and criticisms in the preparation of this paper, to the Wisconsin State Board of Health for supplying information on the fluoride content of the water supplies of the seven Wisconsin cities with high dental caries attack rates, and to Principal Statistician Selwyn D. Collins, National Institutes of Health, for a review of the paper.

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DOMESTIC WATER AND DENTAL CARIES, INCLUDING CERTAIN EPIDEMIOLOGICAL ASPECTS OF ORAL *L. ACIDOPHILUS*

By H. Trendley Dean, Philip Jay, Francis A. Arnold, Jr, Frank J. McClure, and Elias Elvove

INTRODUCTION

A recent report (*1*) has pointed out an inverse relationship between endemic dental fluorosis and dental caries. An increased freedom from dental caries was demonstrable in school children with verified continuity of exposure to domestic waters the fluoride content of which measured 1.7 to 2.5 parts per million, when compared with children of similar ages living in areas where the domestic water supply contained relatively low amounts of fluorides (0.6 to 1.5 p.p.m. F). These observations permitted the formulation of the hypothesis that the factor or factors responsible for partially inhibiting the development of dental caries was present in the domestic water supply and, also, was operative whether or not the tooth showed macroscopic evidence of mottled enamel. Further support was given to this hypothesis by a study of the data on dental caries reported in Public Health Bulletin No. 226 (*2*), the analysis disclosing a lower amount of dental caries in mottled enamel areas than in nonendemic areas in the same State.

To test further the hypothesis a detailed survey of four Illinois cities was planned: Galesburg and Monmouth, where the communal water supplies contain 1.8 and 1.7 parts per million of fluorides, respectively, and the nearby cities of Macomb and Quincy, where the common water supplies are relatively free from fluordies (0.2 p.p.m. F). At Galesburg and Quincy stimulated saliva samples were collected from approximately 200 children in each city and a quantitative estimation of the *L. acidophilus* was made. Saliva samples from 145 children of these two groups were forwarded to the National Institute of Health for a determination of the amylolytic activity. The purpose of the survey was to study the possible relationship of the domestic water supply to the amount of dental caries in each community.

It is well known that factors such as age, sex, and color influence the amount of dental caries in a given group of children. The extensive experimental work in the field of nutrition, including controlled human experiments, points to diet as a factor of major importance, especially in its relation to a high or low carbohydrate intake. Among other factors, latitude and intensity of sunlight have been suggested. In the planning of this survey an attempt was made to take account of these factors insofar as possible. The plan was, briefly, to limit all clinical examinations to 12-, 13-, and 14-year-old white children with continuity of risk of exposure to the variable being studied. It was thought that the magnitude of the sample at Galesburg and Quincy and the inclusion of practically all children possessing the requisites of continuous exposure at Monmouth and Macomb would largely equalize dietetic variations within the groups. The proximity of the cities themselves, located as they are in the flat prairie country of the western part of north central Illinois, should largely eliminate differences in sunlight intensity. Specific discussion of these factors and the methods adopted in an attempt to equalize them within the limits of a survey of this nature will appear later in the text.

Before describing further the methods used in the study and the findings, it seems desirable to record certain basic information, including population and climatological data, and a description of the physical set-up of the four water supplies concerned

Population of cities studied.—Population statistics for the 4 cities studied are given in table 1. Briefly, the population of Galesburg, Monmouth, Macomb, and Quincy in 1930 was approximately 88, 91, 97, and 93 percent, respectively, native white.

Extrinsic factors (hardness of the domestic water, latitude, and intensity of sunlight).—Mills (*3*) states that dental caries shows certain definite relationships to extrinsic factors such as, for example, latitude, sunlight intensity, and hardness of the drinking water. The reports of Förberg (*4*) in Sweden, Röse (*5*) in Germany, Cook (*6*) in England, and Mills (*3*) with respect to the United States, indicate that an increased hardness of the drinking water is associated with a lower amount of dental caries. The limited data shown in an earlier report (*1*) by one of us (H. T. D.), together with unpublished data on other cities mentioned in that article, fail to disclose a consistent relationship between the hardness of the domestic water supply and the amount of dental caries when comparisons are made among communities in the same geographical region. It seems that carefully conducted epidemiological studies will be required

Table 1.—*Stability and composition of population of 4 Illinois cities (Galesburg, Monmouth, Macomb, and Quincy), according to the census of 1930*

	Galesburg	Monmouth	Macomb	Quincy
	POPULATION			
1910	22,069	9,128	5,774	36,587
1920	23,834	8,116	6,714	35,978
1930	28,830	8,666	8,509	39,241
	COMPOSITION OF NATIVE WHITE POPULATION, 1930			
Total	25,458	7,862	8,267	36,601
Native parentage	19,556	6,647	7,604	27,910
Foreign or mixed parentage	5,902	1,215	663	8,691
Foreign-born white	2,213	366	122	1,461
	NATIVE WHITE OF FOREIGN OR MIXED PARENTAGE BY COUNTRY OF BIRTH OF PARENTS [1]			
Total	5,902	1,215	663	8,691
England	489	(2)	(2)	306
Irish Free State	707	(2)	(2)	524
Sweden	2,850	(2)	(2)	(3)
Germany	701	(2)	(2)	6,882

[1] Countries represented by less than 200 are omitted.
[2] Not given in census statistics for cities under 10,000 population.
[3] Less than 200.

Table 2.—*A 5-year summary of available data concerning number of clear, partly cloudy, and cloudy days recorded for the 4 cities studied, and the elevation of each*

	Elevation (feet)	Number of days														
		Clear					Partly cloudy					Cloudy				
		1937	1936	1935	1934	1933	1937	1936	1935	1934	1933	1937	1936	1935	1934	1933
Galesburg	758	189	223	(1)	178	(1)	60	50	(1)	73	(1)	116	93	(1)	114	(1)
Monmouth	763	137	168	119	150	160	93	95	96	106	96	135	103	150	109	100
Macomb	702	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
Quincy	488	174	207	143	185	155	72	67	69	64	73	119	92	153	116	137
State average	....	158	183	138	169	162	92	87	86	92	91	115	96	141	104	112

[1] Not recorded.

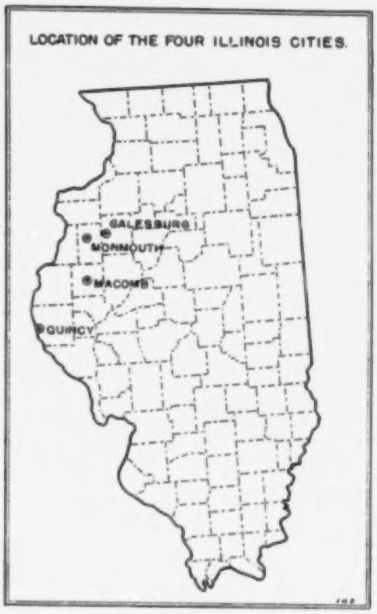


Figure 1

for an answer to this particular aspect of the dental caries problem.

In this study, in order to equalize in so far as possible factors such as latitude, sunlight, and possibly other geographical conditions, cities not far distant from one another were selected for study. Their location is shown in figure 1. Climatological data with respect to sunlight are shown in table 2.

Description of the Communal Water Supplies

Galesburg, Ill.—The public water supply of Galesburg is obtained from two 2,414-foot wells drilled to the Cambrian sandstone. The first well was drilled in 1919, installed in 1920, and has been in continuous use since. In 1928 a second well of the same depth was completed. The casing record of both wells indicates that water from the St. Peter sandstone is completely cased off. Since 1928 these two wells have furnished practically all (more than 98 percent) of the water used by the population.

Between 1924 and 1928 the common water supply consisted of approximately 60 percent from the first "Potsdam" (2,414-foot) well and 40 percent from wells in the St. Peter sandstone. The latter wells (central fire station and Brooks Street station) were drilled in 1917 and 1918, and are 1,252 and 1,245 feet deep, respectively. Both of these latter wells are cased to the St. Peter sandstone so as to exclude water from higher levels. Water history prior to 1924 is omitted, since this would be prior to the year of birth of the group of children examined and, therefore, not relevant. Since 1934 about 95 percent of the water used has been pumped from the larger of the two "Potsdam" wells, known as the "Thorpe well." Treatment is limited to chlorination, 0.2 of one part per million. Since 1928, therefore, the common water supply has been obtained from one source, the 2,414-foot wells drilled to the Cambrian sandstone.

Monmouth, Ill.—The municipal water supply of Monmouth is obtained from two wells, 2,445 feet in depth. The first well was completed early in 1925, the second in 1926. Both wells obtain their water from the "Potsdam" stratum of the Cambrian sandstone. During the first 6 or 8 months of 1925 some water from the old wells in the St. Peter sandstone was added to that obtained from the first well, temporarily constituting a mixed supply. The percentage of the municipal water obtained from the old wells is not known, as the pumping records were not available. The second Cambrian well was completed early in 1926, and since that date all municipal water has been obtained from these wells.

Both wells have 90 feet of 20-inch copperoid casing, 400 feet of 18-inch cast-iron casing, and 1,200 feet of 12-inch cast-iron casing. Water from the St. Peter sandstone, which is found between 1,100 and 1,250 feet in this locality, is apparently cased off. No strainers which permit mixture of water from higher strata are present in the casing. There has been practically no change in the water level of either well since installation. Water from these two wells is more than ample for municipal needs; one well is pumped during the day and the other during the night.

Macomb, Ill.—The Macomb public water supply is obtained from two sources. The older is from the Lamoine River located near the water plant. This supply is from a small storage reservoir having an estimated storage of 2.5 million gallons and a drainage basin area of 112,000 acres. This reservoir is formed by a small channel dam in the stream. The newer supply is from Spring Lake about 5 miles northwest of the city limits, which is an impounded reservoir having a surface area of 85 acres, an estimated storage of 150 million gallons, and a drainage basin of 13,000 acres. The water purification plant was put in operation in the spring of 1911 and water has been obtained from the Lamoine River since that time. Because of the periods of low water in the stream and possible water shortage at times, the Spring Lake Reservoir was constructed in 1927. Water from either of these two sources passes through an over-and-under baffle mixing basin having a retention period of 22 minutes based upon the total plant capacity of 1.5 million gallons per day. Alum, 1–15 grains per gallon, is added for the purpose of coagulation in the mixing basin. From the mixing basin the water passes to a settling basin having a detention period of 2.8 hours. Following this treatment the water passes through the sand filters and thence to the clear well and distribution system. Post chlorination with liquid chlorine following filtration is provided at all times. For the control of red water and corrosion, hydrated lime is added to the filter influent. Activated carbon is also applied when necessary to the water before filtration for the removal of tastes and odors.

Quincy, Ill.—The Quincy common water supply is obtained from the Mississippi River. Water from this source has been in constant use for a period considerably in excess of the maximum age of the group examined. Since 1914 the plant has been operated as follows: The raw water from the Mississippi River passes through an over-and-under baffle mixing basin. Dependent upon raw water conditions, alum, 2–9 grains, is added for the purpose of coagulating suspended matter; detention in mixing basin ranges from the 12 to 15 minutes. From the mixing basin, water passes to a settling basin with a detention period of 1½ hours. It then passes to a second mixing basin where lime is added for softening, then to a clarifying basin for a 2½-hour detention, and then to a recarbonation basin where the carbon dioxide obtained from flue gas removes excess lime. The water is then treated with ammonium sulfate and chlorine, which provide disinfection by monochloramine, after which it passes to a settling basin with a 2.6-hour detention. Following this treatment, the water passes to a rapid sand filter, then to the clear well and the distribution system. Post chlorination with liquid chlorine following filtration is provided for when necessary.

PLAN OF STUDY

The study was undertaken primarily for the purpose of obtaining further information on the differences in the amount of dental caries associated with the use of public water supplies of two dissimilar types. The plan of the study called for a comparison of Galesburg and Monmouth with Macomb and Quincy. Consequently every effort was made to have both groups as nearly alike in all relevant respects as was possible. The Galesburg and Monmouth water supplies contained about 1.8 and 1.7 parts per million of fluorides, respectively, and are producing a mild degree of mottled enamel in an appreciable percentage of those using the water during the period of susceptibility to endemic dental fluorosis. The water supplies of Macomb and Quincy are practically free from fluorides, showing only about 0.2 part per million. For the purposes of this study the group of children examined was deliberately limited to children whose permanent teeth were continuously exposed to the influence of the water under investigation.

The study was further limited to 12-, 13-, and 14-year-old white public school children, age being defined by last birthday. Selection of this segment of the school population permits the examination of a group in whom a high percentage of the permanent teeth have erupted. The results of an examination of school children of higher ages than these may be questioned from the standpoint of the representativeness of the sample because of the increasing percentage of children in the higher age groups not attending school.

The group examined was selected in the following manner. Each classroom or assembly hall was visited and the purpose of the survey explained to the children. Those children who stated that they had lived in the city continuously since birth and had always used the common water supply for domestic purposes (drinking and cooking) were assembled in a separate group. This group was then further questioned to determine whether there had been any breaks in the continuity of their residence and water consumption. If questioning elicited information which disclosed breaks in the continuity of exposure (30 days in any calendar year excepted) the child was eliminated from further study. Those remaining constituted the group continuously exposed since birth to the effects of the local water supply. In a second group were included those children born elsewhere but who had entered the community prior to 6 years of age and had resided there continuously since. In this group the permanent teeth had calcified to a varying degree while using other water supplies, but in practically all instances the permanent teeth since eruption had been exposed to no other domestic water than the one being studied.

Because of the fewer number of children available at Monmouth and Macomb, all children falling in one or the other of the above classifications were examined. These two divisions were likewise made in the Galesburg study in order to ascertain whether or not there was any appreciable difference in the amount of dental caries in children whose permanent teeth had calcified while using a water containing 1.8 parts per million of fluorides and those whose teeth had apparently largely calcified while using waters relatively low in fluorides. In this group were 76 children whose histories indicated continuous exposure to the Galesburg common water from at least 6 years of age to the time of the examination. This group, however, had calcified their premanent teeth to a varying degree while using water other than the Galesburg supply. The subsequent analysis of these data revealed so many variations in water histories that break-down of these data were not statistically possible. Since there was no apparent reason for making this classification at Quincy and in view of the larger number of children available, examinations in that city were, in the main, largely confined to children with a history of continuous use of the municipal water from birth.

Immediately after the selection of the group to be studied, and before leaving the classroom or assembly hall, the name, address, age, grade, continuity of residence and other pertinent data were recorded on a sampling card, one being made out for each child.

It might also be noted that each child was further questioned at the time of the clinical examination, 1 to 4 days later, regarding water history. This second cross-questioning occasionally revealed discontinuities in water history

School .... Serial No. ......

Name in full .... (last) .... (first)

Date of birth ....

Birthplace.. (city)

Age... (years and months)

Grade .... Room or class ....

Home address ....

Have you lived here continuously all your life? (check one) .... (yes) .... (no)

If *not*, how old were you when you moved here? .... (years)

Have you lived here continuously since moving here? ....

Sampling card.

of early life but the number eliminated by this second questioning was relatively small.

Specific comments with regard to the sampling in each of the four cities follow:

Galesburg.—In Galesburg there are three junior high schools covering the seventh, eighth, and ninth grades. The study was conducted at two of these schools (Hitchcock and Lombard). On the day of sampling there were present in the three junior high schools 1,059 12-, 13-, and 14-year-old children. About 70 percent, or 743, were pupils of the two schools where the study was made. Of these 743, cards were made out for 369 pupils, 319 of whom were examined. Of the 50 not examined, 33 were children who, on subsequent questioning during the clinical examination, were found to have had breaks in continuity of exposure, while the remainder were children absent on the day of the examination, a few not examined because of lack of time, and colored children who, because of very small numbers, did not warrant a separate classification. Of the 319 children examined, 243 gave a history of continuous use of the Galesburg city water; the remaining 76 showed breaks in continuity prior to 6 years of age but had continuously used the city water since. The 319 children examined represented about 42 percent of the total number of 12-, 13-, and 14-year-old children present in these two schools on the day of sampling.

Monmouth.—At the time of the examination there were enrolled 430 pupils in the sixth, seventh, eighth, and ninth grades of the Monmouth schools. Of these 430 pupils, 148, or about 34 percent, were examined. The 148 examined included all 12-, 13-, and 14-year-old white children in these grades having either of the two requisites of exposure described. Of the 149 children examined, 99 were children who stated that they had used the city water continuously since birth.

Macomb.—At the time of the examination there were enrolled 501 pupils in the sixth, seventh, eighth, and ninth grades of the Macomb public schools. Of this number, 338 were 12, 13, or 14 years of age. In addition, for the purposes of this study, the 60 Macomb children attending the training school of the Western Illinois State Teachers' College were combined with the public school children and together they constitute the group studied at Macomb. There was considerable difficulty in this city in finding an appreciable number of children who had used the city water continuously owing to the common practice of using water from shallow wells (20–25 feet deep) during the summer months for drinking. This custom developed as a result of the impaired palatability of the city water during the summer months because of its higher temperature.

In Macomb 112 12-, 13-, and 14-year-old children were examined. In this group there were 63 whose histories indicated continuous use of the city water supply. Of the remaining 49, 25 were children who had spent part of their early life elsewhere but who had continuously used the city water since at least 6 years of age. These 25 plus the 63 continuous histories, totaling 88, are the only ones actually comparable to those examined at Monmouth. The other 24 examined, children of the ninth grade, were those who had resided in the community continuously but whose history indicated use of the shallow well water each summer. In the sixth, seventh, and eighth grades, children using well water in the summer months were not examined.

Quincy.—At Quincy the eighth and ninth grades of the entire city, composed largely of 13- and 14-year-old children, are consolidated in one large junior high school. In order that there might be an equitable distribution of 12-, 13-, and 14-year-old children in the study group, all 12-year-old children with the necessary requisites of continuity of exposure were examined in 5 of the 12 elementary schools. At our request the superintendent of education selected the 5 elementary schools which in his opinion would give a representative sample of the Quincy school population of that age.

At the Quincy Junior High School on the day of sampling there were 703 12-, 13-, and 14-year-old pupils present. In addition, in the 5 elementary schools there were present 173 12-year-old children, making a total of 876. Sampling cards were made out for 408 children, of whom 306 were examined. Of the 102 not examined, 23 were children who on subsequent questioning during the clinical examination were found to have had breaks in continuity of exposure; 65 were cases not examined for lack of time; and the remainder consisted of absentees and colored children. The greater number of children enrolled at Quincy permitted a high percentage of examinations of children with a history of continuous use of the public water supply since birth; the first 4 days of the clinical examination were devoted almost exclusively to the examination of children falling into this category. Of the 306 children examined, 291 gave a history of continuous use of the Quincy city water. The 306 examined represent 35 percent of the total number of 12-, 13-, and 14-year-old children present in the junior high school and of the 12-year-old children in the 5 elementary schools present on the day of sampling.

Clinical examination.—All examinations were made by a dentist using a mouth mirror and explorer with the child seated facing a window. At Galesburg and Quincy the explorers used were new double-end No. 3; at Monmouth and Macomb double-end No. 6 explorers were used. At all four cities the instruments used in the examination were taken from the sterilizer and placed in a common pool from which the examiner selected the instruments to be used. Failure in coalescence of enamel lobes (pits and fissures) in which the end of the explorer caught but which showed no evidence of caries was not counted as caries. Pits or fissures showing one or more of the following criteria were counted as caries irrespective of how small the cavitation: Slight opacity around the edges, underlying dark stain suggestive of caries, or a perceptible soft feeling when the explorer was inserted in the pit or fissure. Examination of each child consumed approximately 10 minutes' time.

The personal interpretation in diagnosis is subject to some variation between examiners. An attempt was made to equalize this variation by having each of the two examiners examine approximately an equal number of children in each school. Following the collection of the filled out sampling cards, which were well mixed, the cards were numbered serially. One dentist then examined all odd numbered cases, the other, the even numbered. The clinical examinations at Monmouth and Macomb were made by Assistant Dental Surgeon F. A. Arnold, Jr., and Dr. O. S. Hoag, a dentist of the Illinois Department of Public Health; the examinations at Galesburg and Quincy were made by Dental Surgeon H. T. Dean and Dr. O. S. Hoag. The study at Monmouth and Macomb was made in October 1938, that at Galesburg and Quincy during December 1938. The clinical findings were recorded on a schedule form designed for combined dental caries and mottled enamel studies (fig. 2).

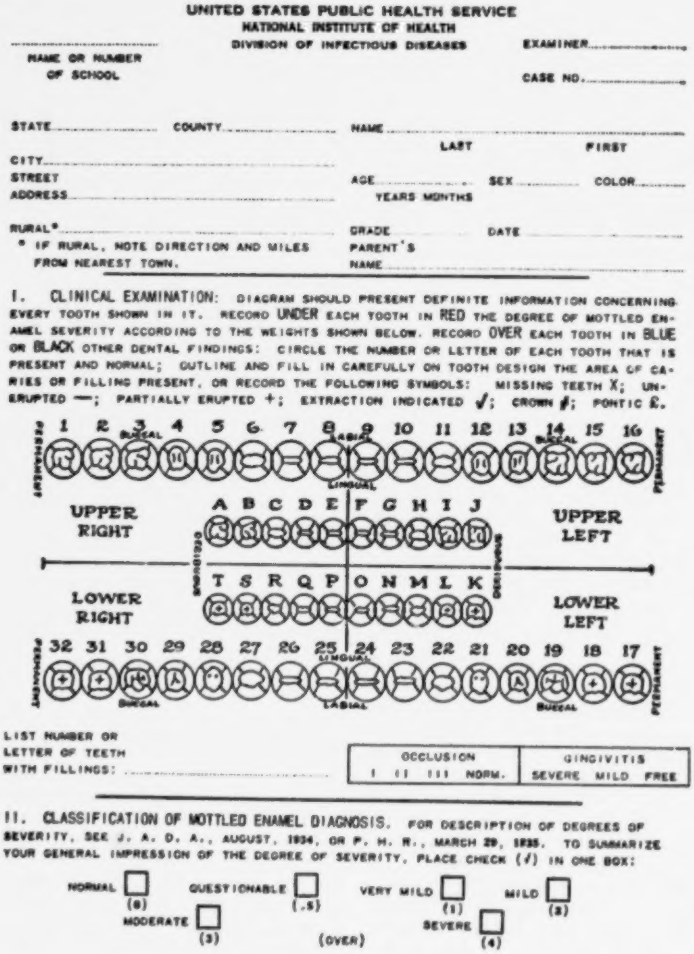
CLINICAL FINDINGS

In the four cities studied 885 children were examined, and they were distributed according to age and sex as shown in table 3.

In table 4 are shown the number of children examined, the number who were caries-free (permanent teeth), the percentage incidence of affection, and the number of carious permanent teeth per 100 children. This table includes: (*a*)

Table 3.—*Distribution of the 885 children examined, according to age and sex*

City	Total	Age in years, last birthday								
		12			13			14		
		M	F	Both sexes	M	F	Both sexes	M	F	Both sexes
	Number									
Galesburg	319	63	60	123	53	61	114	47	35	82
Monmouth	148	23	23	46	29	26	55	20	27	47
Macomb	112	15	22	37	19	26	45	11	19	30
Quincy	306	57	60	117	51	56	107	36	46	82
	Percent									
Galesburg	100.0	19.8	18.8	38.6	16.6	19.1	35.7	14.7	11.0	25.7
Monmouth	100.0	15.5	15.5	31.0	19.6	17.6	37.2	13.5	18.3	31.8
Macomb	100.0	13.4	19.6	33.0	17.0	23.2	40.2	9.8	17.0	26.8
Quincy	100.0	18.6	19.6	38.2	16.7	18.3	35.0	11.8	15.0	26.8


UNITED STATES PUBLIC HEALTH SERVICE
NATIONAL INSTITUTE OF HEALTH
DIVISION OF INFECTIOUS DISEASES

NAME OR NUMBER OF SCHOOL ....
EXAMINER ....
CASE NO. ....

STATE .... COUNTY .... NAME .... LAST FIRST
CITY ....
STREET ADDRESS ....
AGE .... YEARS MONTHS SEX .... COLOR ....
RURAL* ....
GRADE .... DATE ....
* IF RURAL, NOTE DIRECTION AND MILES FROM NEAREST TOWN.
PARENT'S NAME ....

I. CLINICAL EXAMINATION: DIAGRAM SHOULD PRESENT DEFINITE INFORMATION CONCERNING EVERY TOOTH SHOWN IN IT. RECORD UNDER EACH TOOTH IN RED THE DEGREE OF MOTTLED ENAMEL SEVERITY ACCORDING TO THE WEIGHTS SHOWN BELOW. RECORD OVER EACH TOOTH IN BLUE OR BLACK OTHER DENTAL FINDINGS: CIRCLE THE NUMBER OR LETTER OF EACH TOOTH THAT IS PRESENT AND NORMAL; OUTLINE AND FILL IN CAREFULLY ON TOOTH DESIGN THE AREA OF CARIES OR FILLING PRESENT, OR RECORD THE FOLLOWING SYMBOLS: MISSING TEETH X; UNERUPTED —; PARTIALLY ERUPTED +; EXTRACTION INDICATED √; CROWN ♯; PONTIC £.

LIST NUMBER OR LETTER OF TEETH WITH FILLINGS: ....

OCCLUSION	GINGIVITIS
I II III NORM.	SEVERE MILD FREE

II. CLASSIFICATION OF MOTTLED ENAMEL DIAGNOSIS. FOR DESCRIPTION OF DEGREES OF SEVERITY, SEE J. A. D. A., AUGUST, 1934, OR P. H. R., MARCH 29, 1935. TO SUMMARIZE YOUR GENERAL IMPRESSION OF THE DEGREE OF SEVERITY, PLACE CHECK (√) IN ONE BOX:

NORMAL ☐ (0) QUESTIONABLE ☐ (.5) VERY MILD ☐ (1) MILD ☐ (2)
MODERATE ☐ (3) SEVERE ☐ (4)

(OVER)

Figure 2a.—*Face of schedule form.*

Those whose histories indicate continuous use of the city water from birth, and (*b*) all those in group (*a*) plus those who had used the city water continuously since 6 years of age but whose water history between birth and 6 years of age was variable.

In computing an index for showing the amount of dental caries in these population groups, it was decided to express the amount of caries in terms of the number of carious permanent teeth per 100 children examined. The number of carious permanent teeth (both past and present) was determined by combining the aggregates of the number of times the following items were recorded on the clinical examination form: Untreated dental caries, extraction indicated, extracted teeth, and filled teeth. The sum of these four aggregates was then divided by the number of children examined and the quotient multiplied by 100. In computing this rate no single tooth is counted more than once even though one surface may show a carious lesion and another surface a filling. When a single tooth disclosed both a filling and an untreated lesion, the tooth was classified as a "filled tooth."

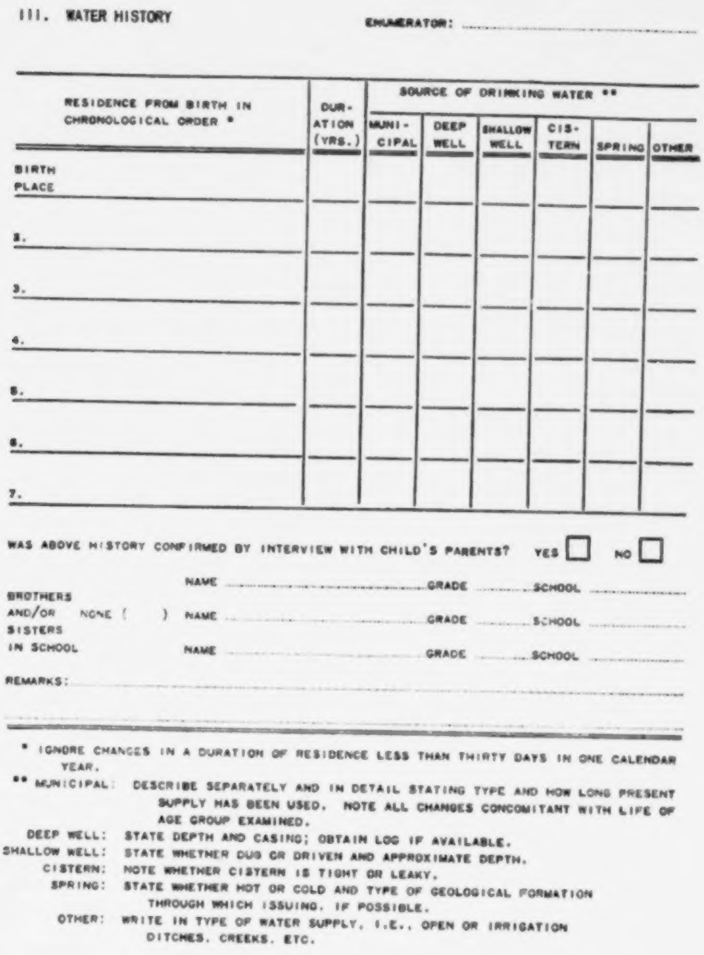
An analysis of the data from this study discloses a remarkable difference in both the percentage incidence of affection and the amount of dental caries present when the observations made in Galesburg and Monmouth are compared with those in Macomb and Quincy. A finding worthy of special comment is the fact that approximately 35 percent of the children examined at Galesburg and Monmouth show no evidence whatsoever of dental caries in their permanent teeth, an usually high percentage for school children of this age group.

With respect to the data shown in section (*b*) of table 4 (all of the children examined in the four cities) it seemed desirable to show how much each of the following items contributed to the rates shown: (1) Untreated dental caries; (2) past dental caries (filled teeth); (3) far-advanced dental caries warranting extraction; and (4) missing permanent teeth (permanent anterior teeth lost because of accident excluded). These data are shown in table 5.

The data shown in section (*b*) of table 5 are also shown graphically in figure 3.

Interproximal caries.—Dental caries in school children is largely divided into two varieties: (*a*) That originating in the pits and fissures, and (*b*) interproximal caries, or that type of smooth surface caries occurring in the neighborhood of the contact point of approximating surfaces.

In this study a striking difference was observed in these two varieties of dental caries, an unusually low amount of in-



III. WATER HISTORY

ENUMERATOR: ....

RESIDENCE FROM BIRTH IN CHRONOLOGICAL ORDER *	DURATION (YRS.)	SOURCE OF DRINKING WATER **					
		MUNICIPAL	DEEP WELL	SHALLOW WELL	CISTERN	SPRING	OTHER
BIRTH PLACE							
2.							
3.							
4.							
5.							
6.							
7.							

WAS ABOVE HISTORY CONFIRMED BY INTERVIEW WITH CHILD'S PARENTS? YES ☐ NO ☐

BROTHERS AND/OR SISTERS IN SCHOOL NONE ( )
NAME .... GRADE .... SCHOOL ....
NAME .... GRADE .... SCHOOL ....
NAME .... GRADE .... SCHOOL ....

REMARKS: ....

* IGNORE CHANGES IN A DURATION OF RESIDENCE LESS THAN THIRTY DAYS IN ONE CALENDAR YEAR.

** MUNICIPAL: DESCRIBE SEPARATELY AND IN DETAIL STATING TYPE AND HOW LONG PRESENT SUPPLY HAS BEEN USED. NOTE ALL CHANGES CONCOMITANT WITH LIFE OF AGE GROUP EXAMINED.
DEEP WELL: STATE DEPTH AND CASING; OBTAIN LOG IF AVAILABLE.
SHALLOW WELL: STATE WHETHER DUG OR DRIVEN AND APPROXIMATE DEPTH.
CISTERN: NOTE WHETHER CISTERN IS TIGHT OR LEAKY.
SPRING: STATE WHETHER HOT OR COLD AND TYPE OF GEOLOGICAL FORMATION THROUGH WHICH ISSUING, IF POSSIBLE.
OTHER: WRITE IN TYPE OF WATER SUPPLY, I.E., OPEN OR IRRIGATION DITCHES, CREEKS, ETC.

FIGURE 2b.—*Back of schedule form.*

terproximal dental caries being present at Galesburg and Monmouth. The examination did not include the use of Roentgen rays, an ancillary aid in detecting small interproximal carious lesions, especially in the posterior teeth. Some appreciation of these differences, however, may be gleaned from a study of the 4 superior permanent incisors. These 4 upper teeth present 8 interproximal surfaces and the amount of dental caries was calculated on the basis of the number of carious lesions per 100 surfaces. Fillings were counted as evidence of past caries. Also these tabulations were made only from the examinations of those children whose histories indicated continuous use of the municipal water throughout life. The findings recorded at Galesburg and Monmouth in contrast to those of Macomb and Quincy are shown in table 6.

These differences are so great that little comment seems necessary. The 2,718 surfaces in the Galesburg and Monmouth children showed only 0.59 carious lesion per 100 surfaces. In the 2,814 tooth surfaces of the Quincy and Macomb children there were 8.9 carious lesions per 100 surfaces, or 16 times as much of this particular variety of dental caries in Quincy and Macomb as in Galesburg and Monmouth.

An unusual freedom from dental caries in anterior teeth was reported by Arnim, Aberle, and Pitney (7) in 1937. This phenomenon, too, was apparently associated with the use of waters containing more than 1.0 part per million of fluoride (F). These workers examined 204 Indian children, aged 7 to 11 years, enrolled in the Government day schools of 6 Rio Grande Pueblo villages in New Mexico and 3 Hopi villages in Arizona. The authors state that no carious lesions were observed in the 1,605 permanent incisors examined, noting further that 24 percent of these teeth had white spots in the enamel, presumably mottled enamel. The chemical analysis of the domestic waters as reported by these authors discloses fluoride concentrations that, in the instance of the New Mexico pueblo, would probably be associated with the production of the milder types of mottled enamel; the analyses from the two Arizona pueblos show concentrations in the neighborhood of the minimal threshold of toxicity, 1.0 part per million of fluoride (F), a concentration that ordinarily produces mottled enamel in about 10 percent of those continuously using it during the susceptible period. The writers note that it is doubtful if "any one child used a single source of water continuously throughout life," a remark showing the uncertainties encountered in correlating clinical observations in nomadic primitive peoples with the use, for an appreciable period of time, of a specific water supply of known mineral composition.

A short time before the above study, Steggerda and Hill (*8*) (1936) reported an unusually low incidence of dental caries in the anterior teeth of Navajo Indians in comparison with Maya In-

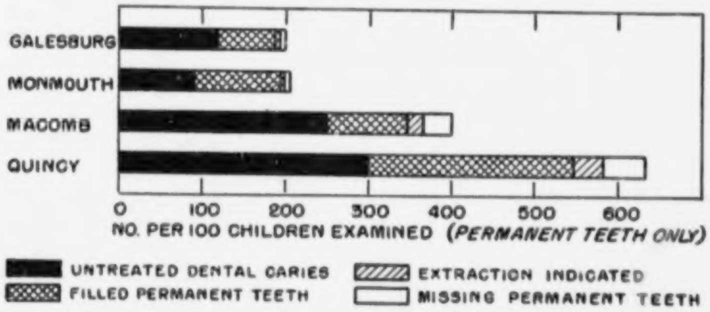


FIGURE 3.—*Distribution of the four signs indicative of past or present dental caries classified according to the number per 100 children.*

TABLE 4.—*Summary of the incidence and amount of dental caries in selected 12- to 14-year-old white children of 4 Illinois cities*

City	Number of children examined	Children with one or more carious permanent teeth		Children with caries-free permanent teeth		Number of carious permanent teeth per 100 children			
						Age in years, last birthday			Total[1]
		Number	Percent	Number	Percent	12	13	14	
(A) 696 CHILDREN WITH HISTORY OF CONTINUOUS USE OF PUBLIC WATER SUPPLY									
Galesburg	243	155	63.8	88	36.2	177	207	201	194
Monmouth	99	63	63.6	36	36.4	115	213	271	208
Macomb	63	54	85.7	9	14.3	315	422	367	368
Quincy	291	279	95.9	12	4.1	563	615	732	628
(B) 885 CHILDREN EXAMINED INCLUDING THOSE IN (A) AND THOSE WHO HAVE CONTINUOUSLY USED THE CITY WATER SINCE 6 YEARS OF AGE; WATER HISTORY PRIOR TO 6, VARIABLE									
Galesburg	319	207	64.9	112	35.1	182	226	196	201
Monmouth	148	96	64.9	52	35.1	150	200	266	205
Macomb	112	96	85.7	16	14.3	346	411	453	401
Quincy	306	294	96.0	12	4.0	567	623	740	633

[1] Inasmuch as the age-sex distribution of the 4 samples (table 3) is relatively uniform, no attempt has been made to adjust for age-sex differences in the rate given as "total" in this and succeeding tables in this paper. This rate, therefore, expresses the amount of dental caries observed in the 12- to 14-year-old children taken as a group.

TABLE 5.—*Summary of the number and the rate per 100 children of carious permanent teeth in 12 to 14-year-old white children of 4 Illinois cities classified on the basis of untreated caries, filled teeth, extraction indicated, or presumably missing because of caries*

City	Number of children examined	Untreated dental caries	Past dental caries (filled teeth)	Extraction indicated	Missing teeth	Total carious permanent teeth
	(A) NUMBER					
Galesburg	319	375	223	20	24	642
Monmouth	148	133	156	4	11	304
Macomb	112	280	109	19	41	449
Quincy	306	917	758	105	156	1,936
	(B) NUMBER PER 100 CHILDREN					
Galesburg	....	117.5	70.0	6.0	7.5	201
Monmouth	....	89.8	105.4	2.7	7.3	205
Macomb	....	250.0	97.3	16.9	36.6	401
Quincy	....	299.6	247.7	34.3	51.0	633

TABLE 6.—*Amount of dental caries in the interproximal surfaces of the 4 superior permanent incisors of selected children of 4 Illinois cities*

City	Number of children examined[1]	Number of proximal surfaces[2]	Number of carious surfaces	Dental caries per 100 surfaces
Galesburg	243	1,934	13	0.67
Monmouth	99	784	3	.38
Macomb	63	502	36	7.2
Quincy	291	2,312	215	9.3

[1] The histories of these children show continuous use of the municipal water throughout life.
[2] Teeth lost by accident excluded.

dians, various racial groups in Jamaica, and a Dutch population in Michigan. The percentage distribution of caries in individual teeth was, in the case of the Navajos, 0.6, 0.6, and 0 for the central and lateral incisors, and cuspids; for the same teeth in the Mayas, the percentages were 17.6, 9.6, and 2.4, respectively.

First permanent molar mortality.—In recent dental literature the term "tooth mortality" has appeared. This appears to be an index of value in measuring certain aspects of the dental caries problem. Recently Knutson and Klein (*9*) reported on the tooth mortality rates computed from the examinations of 4,416 children at Hagerstown, Md., defining tooth mortality as referring to "not only extracted permanent teeth but also those which are indicated for extraction and still present in the mouth." By reason of the relatively large number of children examined, the findings of these workers, reported by sex and single age groups, offer a useful standard for comparative purposes. They found "that the first permanent molars contribute no less than 90 percent of the total mortality for each age and sex group of a representative grade-school population."

For the purpose of determining how closely this index might reflect differences in the amount of dental caries in the four surveyed communities the first permanent molar mortality rate for each community was computed as shown in table 7. In accordance with the definition of the previously cited workers (*9*), first permanent molars listed as "extraction indicated" were combined with those actually missing in computing the rates shown in table 7.

Since tooth mortality rates may be influenced by the amount of remedial treatment received by the child, comparison of the number and percent of filled first molars is desirable. The findings in respect of this point are shown in table 8. There was no record of a school dental program rendering remedial treatment at Galesburg, Monmouth, or Macomb. At Quincy a part-time dentist has been employed by the Quincy public health district since 1922 for rendering dental treatment to any child of school age. This dentist works 3½ hours per morning, 6 days a week, and in 1938 inserted 1,043 fillings in permanent teeth. (*10*).

The incidence of mottled enamel and its relation to the amount of dental caries in an endemic area.—McKay (*11*) has stated that, in spite of their defective structure, teeth with mottled enamel exhibit no greater liability to dental caries than do normally calcified teeth. In a previous article (*1*), one of us (H. T. D.) has stated that "the limited-im-

Table 7.—*Incidence and rate of first permanent molar mortality in 12- to 14-year-old children in 4 selected Illinois cities*

	Galesburg	Monmouth	Macomb	Quincy
Number of children examined	319	148	112	306
Number of first permanent molars missing, or extraction indicated	43	13	57	235
Number missing per 100 children examined	13.4	8.8	50.9	76.8
Percent of those examined with 1 or more missing first permanent molars	9.4	6.7	29.4	37.6

Table 8.—*Number and percent of filled permanent first molars in all children examined in each of the 4 cities*

	Galesburg	Monmouth	Macomb	Quincy
(1) Number of first permanent molars showing past or present caries [1]	443	235	257	994
(2) Number of first molars filled	184	128	72	467
(3) Percent of (1) that are filled	41.5	54.4	28.0	47.0

[1] These totals include not only untreated dental caries and filled teeth but also missing and "extraction indicated," the number of the latter 2 items being shown in line 2, table 7.

munity-producing factor present in the water is operative whether or not the tooth is affected by mottled enamel."

Conditions at Galesburg seemed particularly adapted to further study of this particular aspect of the problem. Here the fluoride concentration of the public water supply is just sufficiently high to produce the milder forms of mottled enamel in about 50 percent of those continuously using the city water, the other 50 percent being free of the macroscopic signs of endemic dental fluorosis.

At Galesburg there were 243 children whose histories indicated continuous use of the municipal water supply. Of this group there were 114 children (46.9 percent) who showed some form of mottled enamel, generally of a very mild type. The remainder, 129, were classified as normal or questionable. Among the 114 children with positive diagnoses of mottled enamel, the number of carious permanent teeth was 200 per 100 children; among the 129 listed as not having mottled enamel, the rate was 186 per 100 children. The factor responsible for the low amount of caries in this city was apparently operative irrespective of whether the child showed macroscopic evidence of mottled enamel or not.

It might also be noted that at Galesburg there was little variation in the incidence of mottled enamel among the three age groups examined. The ninety-one 12-year-old children showed an incidence of 47.2 percent; the eighty-seven 13-year-old children, 42.5 percent; and the sixty-five 14-year-old children, 52.3 percent.

In accordance with a previously described method of computing a community mottled enamel index (*12*) on the basis of the percentage distribution of clinical severity, the approximate mottled enamel index of Galesburg and Monmouth is "slight"; that of Quincy and Macomb, "negative." A survey of Galesburg and Monmouth by one of us (H. T. D.) in 1934 likewise showed an index of "slight." The detailed findings relative to mottled enamel in these four cities as observed in the present survey are shown in table 9.

Bacteriological findings.—At the present time there seems to be a growing belief that the behavior of the oral *L. acidophilus* is a dependable index of the state of dental caries activity.

The fundamental work of the Michigan group (*13*) and others has led to a number of conclusions concerning this group of organisms and its relation to dental caries. Those of seeming major importance in the interpretation of findings dealing with the lactobacilli-caries relationship in any one individual might be restated briefly as follows:

1. Repeated positive cultures of the saliva which consistently show large numbers of *L. acidophilus* are indicative of active caries in a high percentage of cases.
2. Individuals, caries free, or in whom caries is inactive, are characterized by cultures either negative or with organisms present sporadically in low amounts. Repeated bacteriological examinations at weekly or monthly intervals are necessary for the purpose of classifying such cases if the clinical observations are to be correlated with the bacteriological findings.
3. The presence of relatively large numbers of lactobacilli may precede the development of macroscopic dental caries by as much as several months.

Table 9.—*Incidence and distribution of mottled enamel according to the degree of affection*

Macroscopic signs of mottled enamel	Galesburg	Monmouth	Macomb	Quincy	Galesburg	Monmouth	Macomb	Quincy
	Children with history of continuous use of public water supply				Continuous use of public water supply since 6 years of age; variable under 6			
	NUMBER							
Total examined	243	99	63	291	76	49	49	15
Absent:								
Normal	70	6	60	288	32	17	43	15
Questionable	59	26	2	3	16	17	5	0
Present:								
Very mild	82	47	1	0	22	14	[1] 1	0
Mild	29	20	0	0	6	1	0	0
Moderate	3	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0
	PERCENT							
Total examined	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Absent:								
Normal	28.8	6.1	95.2	99.0	42.1	34.7	87.8	100.0
Questionable	24.3	26.3	3.2	1.0	21.1	34.7	10.2	0
Present:								
Very mild	33.8	47.5	1.6	0	28.9	28.6	2.0	0
Mild	11.9	20.2	0	0	7.9	2.0	0	0
Moderate	1.2	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0
Incidence of affection	46.9	67.7	1.6	0	36.8	30.6	2.0	0

[1] During early childhood lived 3 years at Bushnell where the common water supply contains 4.0 parts per million of fluorides.

4. A carious lesion can only be considered active when a subsequent examination demonstrates signs of progression. Attempts to associate the findings of single clinical examinations with single cultures of the saliva oftentimes result in spurious correlations.

In any study on a single individual of the lactobacilli-caries relationship, it is essential that the foregoing conclusions be considered as guiding principles.

The bacteriological aspect of the present study, however, was epidemiological in that the unit of investigation was a population and not an individual. Interest, in other words, was centered on a group and not on an individual. With the development of increasing knowledge regarding oral *L. acidophilus* and its relation to dental caries, it seemed highly desirable to ascertain whether or not oral lactobacilli would reflect the differences in the amount of dental caries in comparable groups of children of two cities with unlike amounts of dental caries. In other words, if a sufficient number of children were sampled in each city, would the *L. acidophilus* counts reflect the amount of dental caries recorded clinically?

In order to learn whether or not group population differences in oral lactobacilli were demonstrable, a "blind test" was carried out at Galesburg and Quincy. Stimulated saliva samples were collected from approximately 200 children (186 at Galesburg; 209 at Quincy) in each city and quantitative estimations of *L. acidophilus* were made.

The bacteriological studies were conducted by Consultant Philip Jay, University of Michigan School of Dentistry, with the assistance of Assistant Dental Surgeon F. A. Arnold, Jr. In order to rule out any unconscious bias based upon a knowledge of the clinical conditions, neither one of the authors engaged in the bacteriological aspects of the study was cognizant of the clinical findings prior to the completion of the bacteriological study. The specimens of saliva were collected from the children in a room separate from the room where the clinical examinations were being made. The bottles used for collecting the saliva were identified by using the serial number assigned to the child's sampling card, the same number likewise appearing on the schedule form upon which the clinical findings were recorded. The clinical examination records were collected twice daily (noon and evening) and remained in the sole possession of one of us (H. T. D.) until the completion of the bacteriological study. Because of the necessary 4-day period of incubation of the plates and broth tubes, the clinical examinations were completed the evening before the first plates were counted.

Upon completion of the bacteriological study, the counts were recorded on the clinical records, the means of identification being the serial number, and at the same time the general clinical condition was cross-recorded on the bacteriological work cards.

For a more detailed analysis of the relationship between the clinical findings and the bacteriological estimations, a separate tabulation has been made of all children for whom both clinical and bacteriological examinations were made. The amount of dental caries in these two groups and the quantitative distribution of the lactobacilli counts are shown in tables 10 and 11.

TABLE 10.—*Summary of the incidence and amount of dental caries in those children for whom a single L. acidophilus examination was made*

City	Number of children examined	Children with one or more carious permanent teeth		Children caries free, permanent teeth		Number of carious permanent teeth per 100 children			
						Age in years, last birthday			Total
		Number	Percent	Number	Percent	12	13	14	
Galesburg	[1] 186	114	61.3	72	38.7	170	192	223	189
Quincy	[2] 209	201	96.2	8	3.8	518	638	745	636

[1] 176 of the group were those with continuous histories since birth.
[2] 204 of the group were those with continuous histories since birth.

TABLE 11.—*Distribution of oral L. acidophilus, Galesburg and Quincy groups*

Estimated number of *L. acidophilus* per cc. of saliva	Distribution of children according to the number of *L. acidophilus* found in saliva			
	Galesburg		Quincy	
	Number	Percent	Number	Percent
Negative	52	28.0	26	12.4
Less than 100	19	10.2	17	8.1
100-1,000	25	13.4	7	3.6
1,000-3,000	10	5.4	5	2.4
3,000-12,000	22	11.8	22	10.5
12,000-21,000	20	10.8	17	8.1
21,000-30,000	10	5.4	6	2.9
30,000 and over	28	15.0	109	52.0
Total	186	100.0	209	100.0

A graphic presentation of the quantitative distribution of the *L. acidophilus* counts is shown in figure 4. It is interesting to note that the amount of dental caries (permanent teeth) in Quincy, 636 per 100 children, is 3.4 times that in Galesburg, 189 per 100 children. While it may be purely coincidental, attention might be called to the fact that the percentage of *L. acidophilus* counts of 30,000 or over is also 3.4 times as high in Quincy as in Galesburg.

Saliva studies (amylase).—Following the streaking of the plates and inoculation of the broth tubes, a number of the samples of the saliva collected on the first day were immediately packed in ice and forwarded to the National Institute of Health for determination of amylolytic activity. The specimens from Galesburg and Quincy arrived at the laboratory at temperatures of 7° and 10° C., respectively. Amylase was selected for study because it is concerned with carbohydrate (starch) degradation in the oral cavity. Considerable uncertainty likewise surrounds the evidence in the literature as to the effect of fluorides on amylase. For details of these saliva studies, the paper by McClure (*14*) should be consulted.

Amylolytic activity of the two groups of saliva was compared in terms of total reducing sugars, calculated as maltose, resulting after a half-hour reaction period of saliva with a 1 percent soluble starch solution as substrate. Optimum conditions for the reaction were maintained by the use of a suitable buffer, and the requisite sodium chloride for enzyme activation.

A total of 63 specimens of saliva from children living in Quincy averaged 105.9 ±5.2 mg. maltose, as compared with an average of 108.7±3.1 mg. of maltose for 82 specimens from children living in

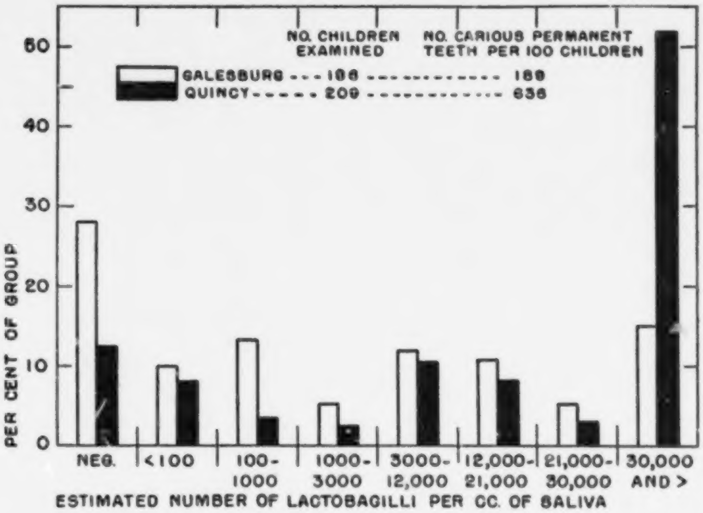


FIGURE 4.—*Percentage distribution of lactobacilli in the saliva of the groups examined at Galesburg and at Quincy, classified according to the estimated amount.*

Galesburg. These results include all the salivas sent for biochemical study for each group, although the individual data indicate that a number of samples included in the above average data may have deteriorated following the time of collection, in spite of the low temperature maintained.

Values somewhat less than 90 mg. of maltose are thought to give evidence of loss of amylolytic activity. Table 12 gives information regarding the variations in the data.

The means of the distribution show no statistically significant differences. Subject, therefore, to possible changes in the saliva owing to the influences of field conditions associated with the collection of these samples, it would appear that the continued ingestion of a domestic water containing, on the average, 1.8 parts per million of fluorine does not change the amylolytic activity of saliva.

Chemical analyses of the common water supplies.—In a previous study 12 consecutive monthly samples of the common water supplies of Galesburg and Monmouth were analyzed. Pub. Health Rep., *50*: 1719–1729 (Dec. 6, 1935). These samples were collected between November 1933 and October 1934. The fluoride concentrations quoted in the present report, 1.8 parts per million for Galesburg and 1.7 parts per million for Monmouth, represent the arithmetic mean of this series. A single sample of each supply collected recently, and reported in table 13, showed a fluoride content of 1.9 p.p.m. for Galesburg and 1.6 p.p.m. for Monmouth, values which, in relation to the mean of the 1933-34 figures, are within the range of the possible experimental error of the method of determination.

The chemical analyses of the waters from Quincy and Macomb should not be accepted literally as representing the average mineral composition of the water used by the inhabitants throughout the year. As in all surface water supplies, seasonal and annual rainfall or other meteorological conditions undoubtedly influence the mineral composition of the water. Twelve consecutive monthly samples are highly desirable in a study of either surface supplies or shallow wells. The mineral composition of water from deep wells, on the other hand, ordinarily shows little fluctuation, the analyses of the samples collected at Galesburg and Monmouth in 1938 being almost identical with those collected in 1933–34.

The chemical analyses of these four samples are shown in table 13; the fluoride content was estimated by means of the zirconium-alizarin reagent (*15*).

The samples of water from Galesburg and Quincy were received in December 1938, and those from Monmouth and Macomb were received in September 1938.

TABLE 12.—*Distribution of the saliva specimens by cities according to maltose-producing activity expressed in milligrams*

Maltose (mg.)	Galesburg		Quincy	
	Number	Percent of total	Number	Percent of total
(45)–50	0	0.0	4	6.3
50-90	12	14.6	7	11.1
90-100	5	6.1	6	9.5
100-110	16	19.5	11	17.5
110-120	27	32.9	22	34.9
120-130	16	19.5	7	11.1
130-(137)	6	7.3	6	9.5
Total	82	....	63	....

DISCUSSION

General findings.—Marked differences in the percentage incidence and the amount of dental caries has been demonstrated between groups of 12-, 13-, and 14-year-old white public school children of two Illinois cities, Galesburg and Monmouth, where the common water supply contains 1.7–1.8 parts per million of fluorides when compared with children of like age in two other nearby Illinois cities (Quincy and Macomb) where the public water supply is practically free of fluorides (0.2 part per million).

While on the basis of our present knowledge it appears reasonable to associate the low caries rates observed at Galesburg and Monmouth with the presence of small amounts of fluorides in the domestic water, the possibility that the composition of the water in other respects may also be a factor should not be overlooked. The western half of Oklahoma seems to furnish some support for this concept. An analysis of the dental caries rates of this State as reported in Public Health Bulletin No. 226, together with observations made by one of us (H. T. D.) in connection with mottled enamel studies, indicate that the dental caries rates are appreciably lower in that part of the State in general west of the Permian outcropping. The part of Oklahoma seemingly characterized by low dental caries rates lies east of the Texas and Oklahoma Panhandles, a region where mottled enamel is generally endemic and dental caries rates low. The slope topographically is eastward. But the increased freedom from dental caries in this region may not be attributed entirely to fluoride in the water since preliminary tests have indicated concentra-

Table 13.—*Chemical analyses of the common water supply of Galesburg, Monmouth, Macomb, and Quincy, Ill.*[1]

	Galesburg	Monmouth	Macomb	Quincy
	Parts per million			
Residue on evaporation	1,097.2	1,048.0	217.1	159.2
Loss on ignition	91.8	59.0	52.2	30.0
Fixed residue	1,005.4	989.0	164.9	129.2
Silica (SiO_2)	11.3	11.2	3.6	11.2
Iron (Fe)	.04	.1	.07	.02
Aluminum (Al)	0	0	0	.2
Calcium (Ca)	62.2	65.0	47.1	28.2
Magnesium (Mg)	25.3	26.6	15.9	4.5
Sodium and Potassium (calculated as Na)	294.1	250.2	7.5	11.6
Carbonate (CO_3)	0	0	0	6.0
Bicarbonate (HCO_3)	297.6	279.3	178.1	20.7
Sulfate (SO_4)	352.8	407.3	38.3	55.1
Nitrate (NO_3)	3.1	3.3	1.0	3.5
Chloride (Cl)	191.0	114.5	4.0	15.3
Fluoride (F)	1.9	1.6	.2	.2
Phosphate (PO_4)	0	----------	----------	0

[1] Assistant Chemist C. G. Remsburg carried out the determinations other than fluoride, using mostly the methods given in the Standard Methods of Water Analysis of the American Public Health Association. The phosphate was determined colorimetrically by an adaptation of the Benedict and Theis method (J. Biol. Chem., 61: 63 (1924)).

tions of fluoride insufficient to produce considerable mottled enamel. Sporadic instances, however, of very mild mottled enamel have been observed at Lawton, Chickasha, Shawnee, and other localities in this region. This would indicate that small quantities of fluorides have been consumed by these populations. Whether or not concentrations of fluoride insufficient to produce considerable mottled enamel may still be sufficient to lower the dental caries rates remains to be determined. For this reason it seems highly desirable that dental caries studies should be accompanied by complete chemical analyses of the domestic waters, including a search for the comparatively rare elements. This seems particularly applicable in those instances where the presence or absence of fluorides may not entirely explain the observed differences in dental caries rates.

Considering the apparent similarity in the population groups and the method followed in the selection of the samples examined, it is difficult from an epidemiological standpoint to ascribe these differences to any other cause than the common water supply. If this ascription proves correct, the possibility of partially controlling dental caries through the public water supply becomes of more than academic interest.

A brief recapitulation of the major findings of this study follows:

Incidence and amount of dental caries.—In the 319 children examined at Galesburg and the 148 examined at Monmouth, the number of carious permanent teeth per 100 children was 201 and 205, respectively. At Macomb and Quincy, examinations of 112 and 306 children disclosed rates of 401 and 633. In other words, there was approximately twice as much dental caries at Macomb and more than three times as much at Quincy as was observed at Galesburg or Monmouth. It must also be remembered that the amount of dental caries at Quincy is not unusually high (633); it merely appears high in relation to that of Galesburg. According to the data in Public Health Bulletin No. 226(*2*), for the same age groups of white children, the neighboring city of Davenport, Iowa, shows 656 carious permanent teeth per 100 children, Minneapolis and St. Paul, Minnesota, 578 and 590, respectively, while seven Wisconsin cities using common water supplies low in fluorides, previously cited in another article (*1*), show rates ranging from 646 to 917. The differences in the percentages of children caries-free with respect to their permanent teeth is of additional interest. At Galesburg and Monmouth about 35 percent of those examined were caries free; in Macomb and Quincy, only 14 and 4 percent, respectively, were free from dental caries.

Interproximal or smooth surface caries.—An unusual difference noted was that in interproximal, or smooth surface, caries. Using the 8 surfaces of the 4 superior permanent incisors for illustrative purposes and limiting the comparisons in all 4 cities to only those children who used the city water supply continuously throughout life, the 2,718 surfaces in Galesburg and Monmouth showed only 0.59 carious lesions per 100 surfaces. In Macomb and Quincy, in 2,814 surfaces there were 8.9 carious lesions per 100 surfaces, or 16 times as much of this particular type of dental caries in Macomb and Quincy as in Galesburg and Monmouth. Such differences are arresting in their relation to the study of the genesis of dental caries. Is a markedly increased vigor in the exciting cause of dental caries required for the development of dental caries on smooth surfaces in contradistinction to that originating in pits and fissures where a focus of carbohydrate degradation is provided for by the failure in coalescence of the lobes of enamel? These surprising differences, demonstrable in school children, recall a recent report of Rosebury (*16*) who, in a study of experimental dental caries in rats, differentiates between occlusal fissure caries and proximo-gingival caries in the rat molars. This latter lesion he considers analogous to proximal caries in man. In the rat the proximogingival type of lesion is produced under conditions comparable to those instrumental in producing the occlusal fissure type but with a markedly lower frequency, and as Rosebury remarks "under conditions that suggest a somewhat distinctive etiology."

Relation between mottled enamel and dental caries.—At Galesburg where the approximate community mottled enamel index is "slight," there was no significant difference in the amount of caries between those children with mottled enamel and those without. The amount of dental caries in the 114 children with mottled enamel was 200 per 100 children examined; in the 129 listed as not having mottled enamel, it was 186 per 100 children. It would appear that the factor responsible for the low amount of caries in this city was operative irrespective of whether the child showed macroscopic evidence of mottled enamel.

Bacteriological findings.—Quantitative estimation of the amount of oral *L. acidophilus* in the saliva of 186 children in Galesburg and 209 children at Quincy was made. The group population differences in the oral lactobacilli closely reflected the differences in the amount of dental caries. The percentage of bacteriological counts of 30,000 or over was 3.4 times higher in Quincy than in Galesburg, a ratio equivalent to the differences noted clinically in the same children, 636 to 189 carious permanent teeth per 100 children.

Saliva studies.—A study of saliva specimens from 63 children in Quincy and 82 from Galesburg showed no significant difference in the averages between the two groups in the rate of amylolytic activity.

SUMMARY

1. Two Illinois cities (Galesburg and Monmouth), using a domestic water closely similar in source and mineral composition, show similarly low dental caries rates, 201 and 205 carious permanent teeth per 100 children, respectively.

2. Two nearby cities (Macomb and Quincy), using a domestic water dissimilar in type and mineral composition

from that of Galesburg and Monmouth, are characterized by dental caries rates double and treble those observed at Galesburg and Monmouth.

3. The Galesburg and Monmouth water supplies contain 1.8 and 1.7 parts per million of fluoride (F), the Macomb and Quincy waters only 0.2 part per million. While it seems reasonable to associate the low dental caries rates with the higher fluoride content of the communal water supplies, the possibility that the composition of the domestic waters, other than the fluorine content, may be a factor should not be overlooked.

4. Using the approximal surfaces of the 4 superior incisors as a basis of measurement, there was 16 times as much interproximal caries in Macomb and Quincy as in Galesburg and Monmouth.

5. The amount of *L. acidophilus* in the saliva closely reflected the difference in the dental caries rates between Galesburg and Quincy. Bacteriological studies were not made at Monmouth and Macomb mainly because of the smaller number of children available for study in these two cities.

6. The quantity of amylase secreted in the saliva disclosed no group population differences between Galesburg and Quincy.

7. From an epidemiological standpoint, it is difficult to ascribe these differences to any cause other than the common water supply.

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DOMESTIC WATER AND DENTAL CARIES. I. A Dental Caries Study, Including *L. Acidophilus* Estimations, of a Population Severely Affected by Mottled Enamel and Which for the Past 12 Years Has Used a Fluoride-Free Water

By H. TRENDLEY DEAN, PHILIP JAY, FRANCIS A. ARNOLD, JR., and ELIAS ELVOVE

Epidemiological evidence points to an inverse relationship between endemic dental fluorosis (mottled enamel) and dental caries (*1, 2, 3, 4*). Recent reviews (*5, 6*) have discussed many of the epidemiological, biochemical, bacteriological, and experimental aspects of this phenomenon, but its mechanism is not, as yet, clear. Whether or not this inhibitory agent operates locally, systematically, or even involves structural or compositional factors is still undetermined. Recent studies (*7, 8, 9, 10, 11, 12, 13*) shed some light on certain aspects of the essential points involved.

For the purpose of possibly clarifying several of these questions, insofar as they relate to human populations, quantitative epidemiological studies were planned, involving (*a*) populations exposed to waters of a relatively high fluoride concentration during the period of tooth calcification followed by exposure, during the posteruptive period, to waters free of fluorides; and (*b*) populations whose teeth calcified while using waters free of fluorides followed. during the posteruptive tooth period, by exposure to waters whose fluoride (F) content exceeded the minimal threshold of endemic dental fluorosis, one part per million.

This report describes a study embodying many of the requisites of this first desideratum. A subsequent paper in this series will record the results of a study in which an almost opposite set of conditions prevailed.

This study was made at Bauxite, Ark., a community which has occupied a prominent place in mottled-enamel history. In 1928 Kempf and McKay (*14*) conducted a mottled-enamel study of this community and recorded an incidence of affection of 100 percent. The public water supply at that time was obtained from deep wells and was subsequently found to contain one of the highest fluoride concentrations of any known common water supply in the United States, close to 14 parts per million. As might be expected, an unusually severe type of mottled enamel had developed in the community. A few months after the 1928 survey, Bauxite changed its public water supply from that of the deep wells to a water obtained from the nearby Saline River, a water free of fluorides and which had been used by the nearby city of Benton for a number of years.

1938 BAUXITE SURVEY

Approximately 10 years after the change in water supply, the children of Bauxite were resurveyed by Dean, McKay, and Elvove (*15*). Briefly, their study showed that the older children, whose permanent teeth had calcified while they were using the "old," or deep-well supply, showed moderate to severe types of mottled enamel, while those children born subsequent to the change in the water supply were practically free of mottled enamel. For details of this study the original report (*15*) should be consulted.

The dental conditions of the 106 Bauxite school children (ages 6–15 years) were examined independently by each of the field examiners (H. T. D. and F. S. McK.). In independent dental examinations it is obvious that no two dental examiners will be in complete agreement with respect to all teeth diagnosed as carious. This is especially true in communities characterized by low dental caries experience rates where oftentimes pits and fissures introduce an important problem of subjective assessment. Table 1 therefore reports only those teeth on which both examiners were in agreement. All examinations were made with the aid of mouth mirrors and explorers. In addition to the enamel hypoplasia characteristic of endemic dental fluorosis (mottled enamel), other dental pathology such as dental caries (treated and untreated), missing teeth, and types of enamel hypoplasia other than mottled enamel, were recorded. Following the clinical examination, the home of each child was visited and the parent interviewed regarding the type of domestic water used by the child throughout life. This house-to-house recheck resulted in the elimination of the schedules of 24 children whose histories indicated the use of a domestic water, other than the Bauxite communal water supply, for a period exceeding 30 days in any calendar year. After the house-to-house check there remained 82 children whose history, confirmed by the parents, indicated continuous exposure to the Bauxite communal water supply.

Because of the complementary nature of the unpublished dental caries data from the 1938 mottled enamel study of Dean and McKay, a brief analysis of their findings, together with a short reference to certain epidemiological constants, is introduced as a means of aiding in a clearer conception of the findings of a study made at Bauxite in April 1940. In the epidemiology of dental caries it is generally accepted that under normal conditions the dental caries experience rates bear a direct relationship to the number of years of exposure to the risk of dental caries. The term "dental caries experience" was introduced by Klein and Palmer (Pub. Health Rep., *55*: 187–205 (Feb. 2, 1940), and earlier articles referred to in this reference) who state that "a reconstitution of the caries experience in the permanent teeth of children may be accomplished with a fair degree of precision by totaling the *mutually exclusive* numbers of carious teeth (irrespective of the number of defects per tooth), the number of filled teeth, and the number of extracted teeth plus those indicated for extraction. The summation of these values gives a count of the number of permanent teeth showing evidence of having been attacked by caries; * * *." When it is desired to express dental caries experience as a rate per 100 children, the sum of the four aggregates referred to (number of teeth with untreated dental caries, filled teeth, extracted teeth, and those indicated for extraction) is divided by the number of children examined and the quotient multiplied by 100.

In the reference cited above, attention is also called to the fact that teeth with evidence of caries experience have been designated by various other terms. Salzman, for instance, uses the expression "ex-teeth," while Hollander and Dunning refer to them as "affected teeth." Since dental caries is a lesion incapable of self-repair, the dental caries experience rates for any single age group represent the dental caries experience of that group cumulative through the posteruptive period, or period of exposure (*cf.* the number of DMF teeth per 100 cases, table 1 of reference *16*).

The amount of dental caries experienced by deciduous teeth cannot be determined by a single clinical examination with the same quantitative precision as that for permanent teeth, since it is not possible to determine whether missing deciduous teeth were lost because of dental caries, or as a result of normal physiological exuviation. It does not seem illogical, however, to assume that years of posteruptive exposure bear a somewhat similar relationship to the amount of dental caries in the deciduous teeth present as has been observed in the permanent teeth.

An analysis, therefore, was made of the amount of dental caries in the deciduous molars and the first permanent molars of the 82 children with verified continuity of exposure to the Bauxite common water supply. The limited number of children available for study (82), distributed over a wide age group (6–15 years), a period when the permanent dentition is subject to varying changes because of the eruption of the permanent teeth, precludes a computation of dental caries experience rates. In the instance of the 1938 data, therefore, study of the dental caries experience is limited to such constants as the deciduous molars present and the first permanent molars, the results being reported on the basis of the percentage incidence of dental caries experience. All missing deciduous molars were excluded from the tabulations for the reason previously stated, and the percentage incidence of dental caries affection was based upon the number of teeth actually present in the mouth. In a community where the clinical examinations indicate an average amount of reparative dental care (fillings, etc.), it may be assumed that a portion, at least, of the population has recourse to dental practitioners for alleviation of their dental needs. Under such conditions it is possible that certain deciduous molars with advanced dental caries may have been extracted by a dentist prior to the time that the tooth would have been normally exuviated. In these 82 children, however, not a single instance of a filled tooth, deciduous or permanent, was noted and one would seem warranted in assuming that in this particular group practically all missing deciduous molars were lost as a result of normal physiological exuviation. Missing permanent first molars, of which there were two, were assumed to have been lost as a result of dental caries; three other first permanent molars showing extensive carious involvement were diagnosed as "extraction indicated."

The dental caries findings of the 1938 study are summarized in table 1. While the number of observations is small there seems to be an inverse relationship between the amount of dental caries and exposure to the "old" common water supply with the high fluoride content. The Bauxite population prior to May 1928 had been exposed to a fluoride intake of unusually high concentration and it would seem that the physiological effects, insofar as they relate to the inhibitory influence on dental caries, had carried over for several years after the change to the fluoride-free river water.

In table 1 the incidence of dental caries in deciduous molars does not show the cumulative increase concomitant with increasing years of exposure. It actually reverses this trend; the highest incidence is observed in the youngest age group, but this group is farthest removed in point of time from the influence of the "old" high fluoride domestic water.

With approximately 9 years of exposure to the risk of dental caries, only 6 deciduous molars were carious, or 9 percent of the 66 deciduous molars present in the 17 children born prior to the change in the water supply. In 21 children born within a year or two after the change to the river water and whose deciduous molars had approximately 6½ years of exposure to the risk of dental caries, 31 were carious, or about 22 percent of the 141 deciduous molars present. And in 19 children born 3 and 4 years after the water change and with the shortest period of exposure to the risk, approximately 4½ years, 52 of these teeth showed carious involvement, or about 36 percent of the 143 deciduous molars present. The incidence of dental caries in the first permanent molars in the group born prior to the change in the water supply was no greater than that recorded in the group born subsequent to the change, irrespective of the fact that the exposure in the former group was nearly four times that of the latter group.

TABLE 1.—*Summary of dental caries findings in 82 Bauxite (Ark.) children with verified continuity of exposure to the common water supply according to the 1938 survey of Dean and McKay*

	Born subsequent to water change				Born prior to water change						Total
Age	6	7	8	9	10	11	12	13	14	15	------
Number of children	5	14	8	13	5	7	6	10	7	7	82
	Deciduous molars[1]										
Number of children with deciduous molars present	5	14	8	13	5	5	3	2	1	1	[3] 57
Number of deciduous molars present in which both examiners' diagnoses agreed	39	104	59	82	27	[2] 16	9	10	2	2	[3] 350
Number of deciduous molars with dental caries experience[1]	14	38	11	20	4	2	0	0	0	0	89
Percent with dental caries experience	36		22								------
	29				9						
Approximate number of years of exposure to risk of caries	4.7		6.6		9.2						------
	First permanent molars										
Number of first permanent molars in which examiners' diagnoses of caries agreed	18	50	28	40	18	25	16	36	24	26	[4] 281
Number of first permanent molars with dental caries experience	2	27	14	17	3	5	6	21	19	9	[4] 123
Percent with dental caries experience	42		46		24			57			
	44				43						
Approximate number of years of exposure to risk of caries	----	1	2	3	4	5	6	7	8	9	------
	1.7				6.7						
Mottled enamel diagnosis of those first permanent molars referred to above:											
Number	18	50	28	40	18	25	16	36	24	26	------
Negative	18	46	28	35	18	20	7	7	0	0	------
Questionable	0	2	0	3	0	4	2	3	0	0	------
Mottled enamel	0	2	0	2	0	1	7	23	23	26	------
Missing, no diagnosis, etc	0	0	0	0	0	0	0	3	1	0	------

[1] Missing deciduous molars excluded from these tabulations.

[2] Two of these molars were diagnosed as "questionable" mottled enamel; no positive diagnoses of mottled enamel were made in any of the deciduous molars in this entire group.

[3] In these 57 children, 368 deciduous molars were present. Both examiners were in agreement as to the presence or absence of dental caries in 350, or approximately 95 percent, of the cases.

[4] In the 82 children in this group there were 328 first permanent molars. For purposes of determining dental caries experience, two molars extracted because of caries were included in the total. The examiners' diagnoses were in agreement in 281, or approximately 86 percent, of the cases.

N. B: Ages 2½ years for deciduous molars and 6½ years for first permanent molars were selected as the time of eruption upon which years of exposure to risk were computed. The limited number of observations did not warrant a separate break-down for the first and second deciduous molars.

Statistical analyses of the differences in the observed rates of the 1938 survey, and also the 1940 study, were made and are shown in tables 1A and 2A. Attention is called to the fact that the critical ratios as shown apply to the observed caries experience only. In interpreting these ratios the greater or lesser years of exposure to the risk of dental caries should also be considered as an important variable.

1940 BAUXITE SURVEY

During the past two years much attention has been focused on the probable relationship of fluorine to dental caries. Continuing epidemiological studies have shown a markedly lessened prevalence of dental caries in communities where the fluoride (F) concentration of the public water supply is slightly over the minimal threshold for endemic dental fluorosis, one part per million.

Although the number of school children available for study at Bauxite was limited, this community seemed to offer some possibility of studying the mechanism of this phenomenon under the conditions of changed exposure to domestic waters differing widely in fluoride concentration. Primarily, the study was planned to determine the amount of dental caries in a population having a moderate to severe type of mottled enamel but which for the past 12 years had been consuming a domestic water free of fluorides. It seemed important also to include an estimation of the number of oral *L. acidophilus* in the saliva of as many as possible of the children included in the study.

METHOD OF SAMPLING

The population studied was divided in the following manner:

Group 1.—This division constituted the basic study group and consisted of all Bauxite pupils present on the day of sampling who had developed mottled enamel while using the "old" Bauxite water supply. They were mostly pupils in the high school and the higher elementary grades and ranged in age from 14 to 22 years. This group consisted of 50 individuals, 26 of whom gave a history of continuous exposure to the Bauxite common water supply. The remaining 24 were persons who had also developed mottled enamel while using the "old" Bauxite water but whose water histories included minor breaks in continuity of exposure. Attention is particularly called to the fact that all in this group showed a relatively moderate to severe type of mottled enamel. Group 1 is shown in table 2 under section B.

TABLE 1A.—*Test of statistical significance of differences noted in the percentage of deciduous molars showing dental caries experience and first permanent molars showing dental caries experience among Bauxite (Ark.) children born subsequent to and prior to the change in the common water supply.*

[Based on Table 1]

Group	Deciduous molars			First permanent molars		
	Percentage showing dental caries experience	Is the difference between the indicated pairs of percentages statistically significant?	Critical ratio [1]	Percentage showing dental caries experience	Is the difference between the indicated pairs of percentages statistically significant?	Critical ratio [1]
1. 40 children born subsequent to the water change (ages 6–9).	29	(1) and (2), yes	4.51	44	(1) and (2), no	0.17
2. 42 children born prior to the water change (ages 10–15).	9	(2) and (1), yes	4.51	43	(2) and (1), no	.17
3. 19 children born subsequent to the water change (ages 6–7).	36	(3) and (4), yes	2.63	42	(3) and (4), no (3) and (5), yes (3) and (6), no	.47 2.20 1.87
4. 21 children born subsequent to the water change (ages 8–9).	22	(4) and (3), yes	2.63	46	(4) and (3), no (4) and (5), yes (4) and (6), no	.47 2.68 1.36
5. 18 children born prior to the water change (ages 10–12).	------	------	------	24	(5) and (3), yes (5) and (4), yes (5) and (6), yes	2.20 2.68 4.28
6. 24 children born prior to the water change (ages 13–15).	------	------	------	57	(6) and (3), no (6) and (4), no (6) and (5), yes	1.87 1.36 4.28

[1] A critical ratio of 2 or more is generally accepted as indicating a difference that is statistically significant, that is, the difference is unlikely to be due to chance. In terms of probability it may be stated that a critical ratio of 2 denotes that a difference as great or greater than the one found may occur by chance only 4.5 times in 100 trials; for critical ratios of more than 2 the probability is even less than 4.5. Thus, when the critical ratio is 2 or more the cause or causes of the difference may be assigned to factors other than chance. (Compare, Pearl, R.: Introduction to Medical Biometry and Statistics, 2d ed., W. B. Saunders Co., Philadelphia, 1938. Table B, page 439.)

Group 2.—This group consisted of 49 children, ages 8–13 years, with a history of continuous use of the Bauxite common water supply. Included were 26 children, ages 8–10, born more than 1½ years after the change in the water supply, and 23 children, ages 11–13 years born within 1½ years of the water change. This latter group of 23 children is designated in table 2, section A, as the "transitional group." Among these 49 children, two cases of "very mild" mottled enamel were observed. For purposes of convenience section A of table 2 also lists older pupils with a history of continuous use of the Bauxite common water supply. The older age group, 14–19 years, shown in section A in composed of the 26 pupils referred to in group 1 as those having continuously used the Bauxite water supply.

Group 3.—In order to have a group roughly comparable in age to group 1 (section B of table 2) all Benton (Ark.) high school pupils with a history of continuous use of the Benton common water supply were examined. There were 45 pupils in this group. As has been previously noted, the Bauxite group who had developed mottled enamel while using the "old" high fluoride water (table 2, section B) had been using Saline River water for the past 12 years only; the Benton group (section C of table 2) had used Saline River water throughout life. No mottled enamel was observed in any of the Benton pupils.

CLINICAL AND BACTERIOLOGICAL FINDINGS

The clinical examinations and the bacteriological estimations were made by Consultant Philip Jay (assistant professor, University of Michigan School of Dentistry) and Passed Assistant Dental Surgeon F. A. Arnold, Jr., United States Public Health Service. A mouth mirror and double end No. 3 explorer were used in making all clinical examinations. These examinations were not made independently as in the case of Dean and McKay (table 1) but each child was examined by the two examiners. When it was questionable as to whether a lesion was to be classified as dental caries or not, agreement between examiners was reached at the time of the examination and the diagnosis in which both concurred was recorded. Comparison of incidences of dental caries in single age groupings or combinations of similar age groupings should not be made between the 1938 and the 1940 survey for the reason that the clinical examinations were made by different examiners. Under such conditions, and especially with regard to the pits and fissures observed in endemic areas of dental fluorosis, the inherent variation associated with subjective assessment results in some individual differences in the diagnosis of dental caries. All mottled enamel diagnoses were made by Passed Assistant Dental Surgeon Arnold. The findings with respect to dental caries experience, using the first permanent molars for comparison, are shown in table 2.

Ordinarily dental caries experience rates are not determined for such small groups, 50 and 45 individuals, or for even much larger groups with such wide age distributions as shown in sections B and C of table 2. But with the reservation implied when dealing with a sample of this nature, the dental caries experience rate, for the permanent teeth, in the 50 Bauxite pupils with mottled enamel was 314 per 100 children; the 45 Benton pupils with no mottled enamel showed a rate of 675. The data upon which these rates are based are shown in table 3.

A previous study (*2*) has shown that the use of a domestic water with a fluoride concentration (1.8 p.p.m.) not greatly in excess of the minimal threshold for endemic dental fluorosis (1.0 p.p.m.) was associated with unusually low oral lactobacilli counts. Whether the observed effects were the result of local, systemic, or even structural or compositional factors in the teeth themselves is not known. Bauxite presented the opportunity of studying bacteriologically a group of children whose teeth were moderately to severely affected by mottled enamel (an enamel which presumably should contain a higher than average fluorine content (*17*)), but who had not used the high fluoride water for the past 12 years. To ascertain whether or not such teeth, with presumably a high fluorine content of the enamel, would be found associated with a lowered number of lactobacilli in the saliva seemed an important step in the attempt to elucidate the manner in which this mechanism operates. For this purpose stimulated saliva samples were collected from 140 of the 149 pupils examined at Bauxite and Benton and quantitative estimations of the number of oral *L. acidophilus* were made by two of the authors (P. J. and F. A. A.). The technique used was similar to that described by Jay (*18*), making equal dilutions of all salivas before plating (1 cc. saliva to 4 cc. broth). The results of the bacteriological study are shown in table 4.

TABLE 2.—*Dental caries findings in Bauxite (Ark.) elementary and high school pupils with and without mottled enamel and exposed to a fluoride-free water for the past 12 years, and in Benton (Ark.) high school pupils exposed to a fluoride-free water throughout life. (Survey made April 22–27, 1940)*

Age in years, last birthday	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
A. 75 BAUXITE (ARK.) PUPILS WITH HISTORY OF CONTINUOUS USE OF COMMON WATER SUPPLY																
	26 pupils using fluoride-free water throughout life A-1			23 pupils of transitional group [1] A-2			26 pupils using fluoride free water past twelve years only; high fluoride water previously A-3									Total
Number of pupils examined	3	15	8	11	7	5	3	3	6	6	7	1	0	0	0	75
First permanent molars:																
Number (missing included)	12	60	32	44	28	20	12	12	24	24	28	4	----	----	----	300
Number showing dental caries experience	6	47	15	21	9	1	3	3	15	7	10	3	----	----	----	140
Percent showing dental caries experience	65			34			25		46		41					
							39									
Number of pupils, caries free	1	1	2	2	4	4	1	2	0	3	1	0	----	----	----	------
Percent of pupils, caries free	15			43			50		25		13					
							27									
Approximate number of years of risk of exposure to caries	3.2			5.7			10.5									
Mottled enamel:																
Number of pupils with	0	1	0	0	1	0	3	3	6	6	7	1	----	----	----	------
Degree of clinical affection (weighted average) [2]	0	0.1	0	0	0.2	0	2.7	3.7	3.8	3.2	3.7	4.0	----	----	----	------
B. 50 BAUXITE (ARK.) PUPILS SHOWING A RELATIVELY MODERATE TO SEVERE TYPE OF MOTTLED ENAMEL. GROUP CONSISTS OF 26 OF "A-3" ABOVE AND 24 OTHERS WITH MINOR VARIATIONS IN CONTINUITY OF USE OF PUBLIC WATER SUPPLY																
Number of pupils examined							4	8	12	9	9	4	3	0	1	50
First permanent molars:																
Number (missing included)							16	32	48	36	36	16	12	0	4	00
Number showing dental caries experience							3	12	32	11	17	7	0	----	1	83
Percent showing dental caries experience							31		51		37					
							42									
Number of pupils, caries free							2	3	0	4	1	2	3	----	0	15
Percent of pupils, caries free							30									
Approximate number of years of risk of exposure to dental caries							10.8									
Mottled enamel:																
Number of pupils with							4	8	12	9	9	4	3	----	1	------
Degree of clinical affection (weighted average) [2]							3.0	3.0	3.8	3.1	3.8	3.5	3.7	----	4.0	------
C. 45 BENTON (ARK.) HIGH SCHOOL PUPILS WITH A HISTORY OF CONTINUOUS USE OF THE COMMON WATER SUPPLY. (NO CHANGE OCCURRED IN BENTON WATER SUPPLY DURING LIFETIME OF THIS GROUP)																
Number of pupils examined							11	9	10	9	4	0	1	1	0	45
First permanent molars:																
Number (missing included)							44	36	40	36	16	----	4	4	----	180
Number showing dental caries experience							30	27	20	33	13	----	0	1	----	124
Percent showing dental caries experience							71		70		58					
							69									
Number of pupils, caries free							1	1	3	0	0	----	1	0	----	6
Percent of pupils, caries free							13									
Approximate number of years of risk of exposure to dental caries							9.9									
Mottled enamel:																
Number of pupils with							0	0	0	0	0	----	0	0	----	------
Degree of clinical affection (weighted average) [2]							0	0	0	0	0	----	0	0	----	------

[1] The public water supply at Bauxite was changed from deep well to filtered river water in May 1928; the "transitional group" includes those pupils born within 1½ years of the change in water supply (a 3-year period covering 1½ years prior to the change and 1½ years subsequent to the change).

[2] The following weights were given to the diagnosis recorded for each individual: normal, 0; questionable, 0.5; very mild, 1; mild, 2; moderate, 3; severe, 4.

DESCRIPTION AND MINERAL COMPOSITION OF THE BAUXITE AND BENTON COMMON WATER SUPPLIES

The description of the water supply used at present at Bauxite and Benton was supplied by Dr. W. B. Grayson, State Health Officer, Arkansas State Board of Health.

Bauxite.—Since May 25, 1928, the Bauxite common water supply has been obtained from the Saline River. Pumps at the river deliver the water to the purification plant through approximately 6½ miles of 6-inch , and ½ mile of 4-inch pipe. The raw water is discharged into a mixing chamber of the over-and-under baffle type and is coagulated with about 0.3 grain per gallon each of sodium aluminate and aluminum sulfate. After mixing, the water flows into a sedimentation basin; the detention period is from 7 to 8 hours. The settled water then passes through a rapid sand filter to a small clear well from which it is pumped to a covered metal tank. Filter wash water is obtained from a 50,000-gallon covered metal elevated tank. Just after the filtered water leaves the pump a dose of about 0.5 p.p.m. of chlorine is added. The treated water flows from the storage tank to the mains by gravity.

The original domestic water supply of Bauxite was obtained from shallow wells and a few springs. With the increase in population and contamination of certain of these surface supplies, a new water supply became necessary, and in 1909 two 297-foot wells were drilled. One of these wells became partly filled at the bottom from caving, and in September 1925 a new 245-foot well was added. The public water supply was obtained from these two deep wells until the use of the filtered river water began in May 1928. At the time of the 1938 survey the 245-foot well was still being used for industrial purposes; the other well had been abandoned. Water from this deep well may be pumped into the distribution system, but it would have necessitated the repair of a relift pump and manipulation of a one-gate valve. There was no record of this having been done since the installation of the filtered river water in 1928. Practically all of the population uses the common water supply.

Benton.—The Benton public water supply is obtained from the Saline River. The present plant was placed in operation in 1936 but Benton has obtained its public water supply from the Saline River for more than 20 years, a period longer than the lifetime of the group examined. A pump delivers the river water through ⅝ mile of 8-inch pipe to a one million gallon settling tank, which is divided in half so that it may be cleaned and still provide settling facilities. From the settling tank the water flows by gravity through ⅝ mile of 8-inch line to the purification plant. Lime and aluminum sulfate, each in doses of 0.15 to 0.2 grains per gallon, are added in a small mixing chamber which is equipped with a mechanical mixer. From the mixing chamber the water flows under a baffle to the sedimentation basin, the detention period being approximately 6 hours. The water from the sedimentation tank then flows through two rapid sand filters, only one filter being used at a time. The wash-water is obtained from the system and is pumped for the backwash pressure. Liquid chlorine is added as the water leaves the filters for the 100,000-gallon covered clear well. The water

Table 2A.—*Test of statistical significance of differences noted in the percentage (a) of first permanent molars showing dental caries experience and (b) of pupils with caries free, first permanent molars, among certain groups of Bauxite and Benton (Ark.) school children*

[Based on Table 2]

Group	First permanent molars			Pupils examined		
	Percentage showing dental caries experience	Is the difference between the indicated pairs of percentages statistically significant?	Critical ratio[1]	Percentage of pupils, caries free, first permanent molars	Is the difference between the indicated pairs of percentages statistically significant?	Critical ratio
A-1. 26 Bauxite pupils using river water throughout life (ages 8-10).	65	A-1 and A-2, yes	4.56	15	A-1 and A-2, yes	2.25
		A-1 and A-3, yes	3.89		A-1 and A-3, no	1.07
		A-1 and B, yes	3.94		A-1 and B, no	1.57
		A-1 and C, no	.69		A-1 and C, no	.23
A-2. 23 Bauxite pupils of the "transitional group" (ages 11-13).	34	A-2 and A-1, yes	4.56	43	A-2 and A-1, yes	2.25
		A-2 and A-3, no	.73		A-2 and A-3, no	1.19
		A-2 and B, no	1.32		A-2 and B, no	1.07
		A-2 and C, yes	5.81		A-2 and C, yes	2.61
A-3. 26 Bauxite pupils using river water during the past 12 years only; deep well water previously (ages 14-22).	39	A-3 and A-1, yes	3.89	27	A-3 and A-1, no	1.07
		A-3 and A-2, no	.73		A-3 and A-2, no	1.19
		A-3 and C, yes	5.09		A-3 and C, no	1.39
B. 50 Bauxite pupils showing a relatively moderate to severe mottled enamel (includes A-3 plus 24 others with some variation in use of water supply).	42	B and A-1, yes	3.94	30	B and A-1, no	1.57
		B and A-2, no	1.32		B and A-2, no	1.07
		B and C, yes	5.50		B and C, yes	2.08
C. 45 Benton pupils with a history of continuous use of the common water supply. (No changes in this supply throughout lifetime of group.)	69	C and A-1, no	.69	13	C and A-1, no	.23
		C and A-2, yes	5.81		C and A-2, yes	2.61
		C and A-3, yes	5.09		C and A-3, no	1.39
		C and B, yes	5.50		C and B, yes	2.08

[1] See footnote, table 1A.

Table 3.—*Distribution of the dental caries experience, permanent teeth, in the Bauxite group with mottled enamel (section B, table 2) and the Benton group with no mottled enamel (section C, table 2) (1940 survey)*

Place	Number of pupils examined	Untreated dental caries	Past dental caries (filled teeth)	Extraction indicated	Missing	Total dental caries experience
		Number				
Bauxite	50	107	21	0	29	157
Benton	45	179	72	11	42	304
		Number per 100 cases				
Bauxite		214	42	0	58	314
Benton		398	160	24	93	675

is pumped from the clear well to a 75,000-gallon covered metal elevated tank and the distribution system.

Mineral composition.—The mineral composition of the common water supplies, based upon samples collected in 1928, 1938, and 1940, is given in table 5.

DISCUSSION

Because of the limited number of observations possible in a small population, the findings of this study must be interpreted with considerable caution. But it does seem from the data presented that the factor responsible for the marked inhibition of dental caries in areas of endemic dental fluorosis may be operative for a considerable period after the group has ceased using the high fluoride water supply. Whether this factor functions locally, systematically, or both ways, is not known.

A first inspection of table 2 might suggest that those pupils with moderate to severe dental fluorosis are less liable to attack by dental caries than those not so affected. A comparison, for instance, of section B with section C (table 2) shows that in a group roughly comparable as regards age, the 50 Bauxite pupils with endemic dental fluorosis, but using a water for the past 12 years similar to that used by the 45 Benton high school pupils during their entire lifetime, have experienced markedly less dental caries in their first permanent molars, 42 percent and 69 percent, respectively. In the Bauxite pupils 15, or 30 percent, were free of caries in all first molars; at Benton, only 6, or 13 percent, had no dental caries.

But the mechanism associated with this limited immunity from dental caries does not seem to be wholly dependent, if at all, upon the presence or absence of macroscopic dental fluorosis. The "transitional group" at Bauxite, born within 1½ years of the change in the water supply and in whom only one case of "very mild" dental fluorosis was observed, also show an inhibition of dental caries when compared with the younger children. Similar observations by Dean and McKay in the 1938 survey, as shown in table 1, indicate that there was some factor inhibiting the development of dental caries in the deciduous molars of those children in Bauxite who were born prior to the change in the water supply. It does not seem logical to explain this observed difference on the basis of the presence or absence of macroscopic dental fluorosis, since no positive diagnosis of dental fluorosis was made on any of the deciduous teeth in this group. These observations on the deciduous teeth are in accord with the findings of Dean (*1*) on six communities with varying amounts of fluorides in the communal water supplies. The 1940 observations at Bauxite with respect to the first permanent molars are in accord with the results reported by Dean, Jay, Arnold, McClure, and Elvove (*2*) for Galesburg, Ill., where no marked difference was found in the children with dental fluorosis compared to those who were not so affected.

In respect to the bacteriological findings, attention may be called to the following points:

First, the enamel of the teeth of all of the Bauxite pupils, ages 14–22 years (section B, table 2), revealed the distinctive hypoplastic signs of dental fluorosis, with the discreet and confluent pitting symptomatic of the severest forms of mottled enamel being common. The excellent photographs taken by McKay (*14, 15*) attest the severity of the mottled enamel developed by the users of the "old" water supply. The teeth of the 45 Benton students, on the other hand, were free of mottled enamel, and

Table 4.—*Summary of the L. acidophilus findings in the salivas from 41 Benton (Ark.) and 89 Bauxite (Ark.) pupils*

Item		Distribution of children according to the estimated number of *L. acidophilus* per cc. of saliva								
		Negative	Less than 100	100 to 1,000	1,000 to 3,000	3,000 to 12,000	12,000 to 21,000	21,000 to 30,000	30,000 and over	Total
Benton pupils without mottled enamel (ages 14–21 years).	Number	7	4	7	3	3	4	3	10	41
	Percent	17.1	9.7	17.1	7.3	7.3	9.7	7.3	24.5	100.0
Bauxite pupils with mottled enamel (ages 14–22 years).	Number	13	10	0	2	10	3	0	12	50
	Percent	26	20	0	4	20	6	0	24	100.0
Bauxite transitional group—born within 1½ years of water change (ages 11–13 years).	Number	10	4	3	3	1	0	0	2	23
	Percent	43.5	17.4	13.0	13.0	4.4	0	0	8.7	100.0
Bauxite children born 1½ years or more after change in water supply (ages 8–10 years).	Number	4	2	4	3	0	1	2	10	26
	Percent	15.4	7.7	15.4	11.5	0	3.8	7.7	38.5	100.0

Table 5.—*Analyses of the waters used at Bauxite and Benton, Ark., in parts per million*

	[1] 1928		[2] 1938		[3] 1940	
	Bauxite deep-well water	Bauxite filtered water (Saline River)	Bauxite 245-foot deep well	Bauxite filtered water (Saline River)	Bauxite filtered water (Saline River)	Benton filtered water (Saline River)
Total residue on evaporation	1003.0	86.0	958.6	60.0	74.0	78.8
Loss on ignition	43.0	14.0	27.4	10.6	11.2	16.2
Fixed residue	960.0	72.0	931.2	49.4	62.8	62.6
Silica (SiO_2)	18.6	6.0	13.6	5.8	5.0	6.0
Iron (Fe)	1.0	.3	.1	.02	0.04	0.06
Aluminum (Al)			.4	0	0	0
Calcium (Ca)	25.3	17.6	30.9	11.4	14.6	17.4
Magnesium (Mg)	7.0	2.1	8.8	3.7	2.9	3.1
Sodium and potassium (calculated as Na)			333.4	2.9	4.7	3.4
Sodium (Na)	344.6	9.6				
Potassium (K)	9.2	3.4				
Carbonate (CO_3)	1.2	0	0	0	0	0
Bicarbonate (HCO_3)	258.2	63.4	247.6	47.5	62.8	62.2
Sulfate (SO_4)	39.6	15.7	36.0	10.3	8.9	8.1
Nitrate (NO_3)	.3	.03	0	.27	0.11	0.16
Chloride (Cl)	415.9	3.75	389.5	3.0	1.5	1.0
Phosphate (PO_4)			.1	0	0	0
Boron (B)			.8	0		
Fluoride (F)	(4)	(4)	14.1	0	[5] 0	[5] 0

[1] Samples collected in July 1928. [2] Samples collected in March 1938. [3] Samples collected in May 1940.

[4] These 1928 samples were recently analyzed (October 1940) for fluoride content by one of us (E. E.) who found that the deep well water contained 13.0 parts per million of fluorides (F), the filtered river water, 0.1 part per million.

[5] Two additional monthly samples from each locality (Bauxite and Benton), collected in June and July 1940, were also examined for fluoride. The results were negative, as in the May samples. The limit of the sensitivity of the fluoride test employed may be considered as about 0.1 part per million. (Pub. Health Rep., 48: 1219–1222 (Oct. 6, 1933).) Assistant Chemist C. G. Remsburg carried out the determinations other than fluoride and boron, using mostly the methods given in the Standard Methods of Water Analysis of the American Public Health Association. The phosphate was determined colorimetrically by an adaptation of the Benedict and Theis method (J. Biol. Chem., 61: 63 (1924)). The boron determinations were made essentially by the method of Foote (J. Ind. Eng. Chem., Anal. Ed., 4: 39 (Jan. 15, 1932)).

showed a type of calcification commonly considered normal. The percentage of high *L. acidophilus* counts (30,000 and over) in both the Bauxite group and the Benton group was approximately the same. An examination of the number of low counts (0 and less than 100) shows 26.8 percent of the Benton group in this category in contrast with 46 percent of the Bauxite group. This difference borders on statistical significance, but the limited number of observations do not warrant any definite conclusions.

Second, the "transitional group" (those born within 1½ years of the water change) shows the lowest amount of dental caries; the youngest Bauxite group (ages 8–10 years), the highest. An analysis of the difference between the low counts recorded for these two groups indicates a statistically significant difference with a critical ratio of 2.88.

An analysis of the data presented in tables 1 and 2 might suggest that certain physiological effects, at least insofar as they relate to the inhibitory influence on dental caries, had carried over in the Bauxite population for several years after the change from the high fluoride water to the fluoride-free water. This seemingly would apply to both the older group with mottled enamel and to the "transitional group" as well. The possibility of physiological effects being carried over in a population several years after cessation of exposure to waters of high fluoride content obviously requires much additional study. From the standpoint of descriptive epidemiology it seems relevant to note that there is no evidence that the population was actually using a fluoride-free water immediately after the introduction of the filtered river water in 1928. This water, although fluoride-free when analyzed in 1938 and 1940, may possibly have contained very small amounts of fluoride during the first few years of its use because of incrustations in the iron pipes of the distribution system, cooking utensils, etc. As noted in the footnote to table 5, a recent analysis of the sample of the 1928 "filtered river water" received at this laboratory in July 1928, showed a fluoride (F) content of 0.1 part per million. It is not possible, however, to learn whether this sample was taken from a tap on the distribution system or from the treatment plant installed a few months before the collection of the sample. A personal communication, however, from Mr. L. R. Branting, superintendent of the Republic Mining & Manufacturing Co., dated October 25, 1940, states that after checking with the laboratory personnel, it seemed to be the consensus that the 1928 sample of filtered river water was taken from a tap in the laboratory. He notes that the community had been using the filtered river water for several months at the time that the sample was collected and that there would have been no particular reason in going to the filter plant when it was possible to collect the sample at the company's chemical laboratory at the administration building. The influence, if any, of this variable cannot, of course, be precisely assessed on the present evidence; the best possible information now available, however, suggests that the 1928 filtered river water sample was collected from a tap on the distribution system.

Another question that naturally arises is whether or not the enamel of certain teeth, despite the absence of macroscopic dental fluorosis, contains more fluorine than the enamel of similar teeth calcified in a community whose public water supply for a number of years has been free of fluorides. Specifically, these are the deciduous teeth (table 1) calcified under the conditions existing in Bauxite prior to or shortly after the change in the water supply and the permanent teeth of the "transitional group" (table 2). With respect to Bauxite, of course, much of this study material is no longer available for biochemical analyses, but continuing studies of selected communities from the time that the water supply is changed may shed some light on this particular aspect of the question.

In an attempt to interpret these observed effects, particularly as regards the "transitional group," the possibility of a placental or mammary transfer, or both, of the factor or factors associated with the dental caries inhibition, must be given careful consideration. In this connection it might be remarked that the transfer of fluorine by placental or mammary means has been presumed on the basis of epidemiological evidence (*4, 19, 20, 21, 22*) and demonstrated experimentally (*23, 24, 25*), although other workers (*26, 27*) have been unable to demonstrate the mammary transfer of fluoride in cow's milk.

It is apparent that much additional study of this phenomenon is needed to elucidate the manner in which this limited immunity from dental caries operates. The partial inhibition of dental caries, moreover, may be only a part of a larger problem in general physiology relative to the continued ingestion of varying amounts of fluorides by general population groups.

Two findings of this study seem worthy of stressing:

1. The older Bauxite group, all of whom showed a moderate to severe type of endemic dental fluorosis, disclosed markedly less dental caries than a comparable group at Benton who were free of mottled enamel.

2. The cumulative increase in the amount of dental caries experience concomitant with increasing years of exposure to risk did not hold true in the Bauxite school population studied. The reversal in trend of this epidemiological constant suggests the noticeable physiological influence which operated in this population and which was presumably associated with the change in the communal water supply.

SUMMARY

1. Bauxite pupils with moderate to severe mottled enamel and exposed to fluoride-free waters during the past 12 years showed markedly less dental caries than a comparable group of Benton pupils without mottled enamel who had been using a fluoride-free water during their lifetime.

2. This limited immunity from dental caries is seemingly not dependent upon the presence of macroscopic mottled enamel because children born within several years of the change in the water supply and practically free of mottled enamel likewise disclosed a low dental caries experience.

3. The youngest age group at Bauxite —those farthest removed in time from the influence of the "old" high fluoride water—shows the highest dental caries experience in spite of the fact that they had been exposed to risk of caries for the shortest period of time.

4. The *L. acidophilus* counts apparently reflect a difference in caries activity in the several groups studied, a result which is seemingly consistent with the clinical findings in these groups.

5. Teeth moderately to severely affected with mottled enamel showed no tendency to rampant dental caries even though they had been exposed to a fluorine-free water for the past 12 years.

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DOMESTIC WATER AND DENTAL CARIES. II. A Study of 2,832 White Children, Aged 12–14 Years, of 8 Suburban Chicago Communities, Including *Lactobacillus Acidophilus* Studies of 1,761 Children

By H. TRENDLEY DEAN, PHILIP JAY, FRANCIS A. ARNOLD, JR., and ELIAS ELVOVE (Clinical Examinations by David C. Johnston and Edwin M. Short)

Recent studies (*1, 2*) have disclosed marked differences in the amount of dental caries among communities often in close proximity to one another. Considering the apparent similarity of the population groups, especially those in Galesburg, Monmouth, Macomb, and Quincy (Ill.), and the method followed in the selection of the samples, it is difficult from a epidemiological standpoint to ascribe these differences to any cause other than the mineral composition of the common water supply. At the present time both epidemiological and experimental evidence points to fluoride as the factor partially inhibiting dental caries, but the possibility that other constituents of the water may likewise play some role cannot at present be entirely ruled out on the basis of the epidemiological evidence available.

A marked difference in the amount of dental caries was particularly noticeable in the Galesburg-Quincy study. In the latter city the children, using a public water supply practically free of fluorides 0.1–0.2 p.p.m.), had experienced more than three times as much dental caries as had a comparable age group living in the nearby city of Galesburg where the common water supply contains 1.8 p.p.m. of fluorides (F). The continued use of this water, somewhat in excess of the minimal threshold of endemic dental fluorosis (1.0 p.p.m.), was found to be associated not only with a low dental caries rate but also with unusually low oral lactobacilli counts.

A domestic water containing 1.8 p.p.m. of fluorides produces the mildest types of mottled enamel in about 45 to 50 percent of those continuously using it during the period of susceptibility, the remainder showing no macroscopic evidence of the affection. A percentage incidence of affection of this approximate order makes possible a comparison of the amount of dental caries in a group of children having the mildest forms of mottled enamel with a comparable group free of mottled enamel. No significant difference in the amount of dental caries between the two groups was apparent and it appeared that the factor responsible for the low amount of dental caries in that city was operative irrespective of whether the child showed macroscopic evidence of mottled enamel or not.

It is obvious that whatever effect the waters with relatively high fluoride content (over 2.0 p.p.m. of F) have on dental caries is largely one of academic interest; the resultant permanent disfigurement of many of the users far outweighs any advantage that might accrue from the standpoint of partial control of dental caries. On the other hand, the demonstration of such marked dental caries differences as were observed at Galesburg and Quincy made advisable a quantitative study of the influence on dental caries of waters with lower ranges of fluoride concentration. If marked inhibitory influences were operative at concentration levels as low as the minimal threshold of endemic dental fluorosis (1.0 p.p.m.), the findings would be of considerable import.

PRESENT STUDY

The basic objective of this study was to determine how low a fluoride concentration in a public water supply would be found associated with relatively low dental caries rates. The study closely followed that made at Galesburg and Quincy (*2*) excepting that only children with continuous exposure to the public water supply of their respective communities were examined. All examinations were limited to 12–14-year-old white public school children, age being defined by last birthday. Selection of this segment of the school population permits the examination of a group in whom a high percentage of the permanent teeth have erupted. The results of an examination of school children of higher age groups introduces the question of representativeness of the sample because of the increasing percentage of children in the higher age groups not attending school.

The communities selected for study necessarily had to possess the dual requisites of (*a*) a population sufficient in size to permit the selection of an adequate sample of children continuously exposed to the influence of the variable under investigation, and (*b*) a public water supply of the desired fluorine concentration with no serious interfering relevant variables in either its physical set-up or its source during the period concomitant with the life of the group examined.

The selection of cities meeting these requirements would have been difficult but for the extensive studies carried on in 1936 by the Illinois Department of Public Health. In 1937 Weart and Klassen (*3*) reported on the fluoride content of many Illinois common water supplies, 78 communities being listed as having

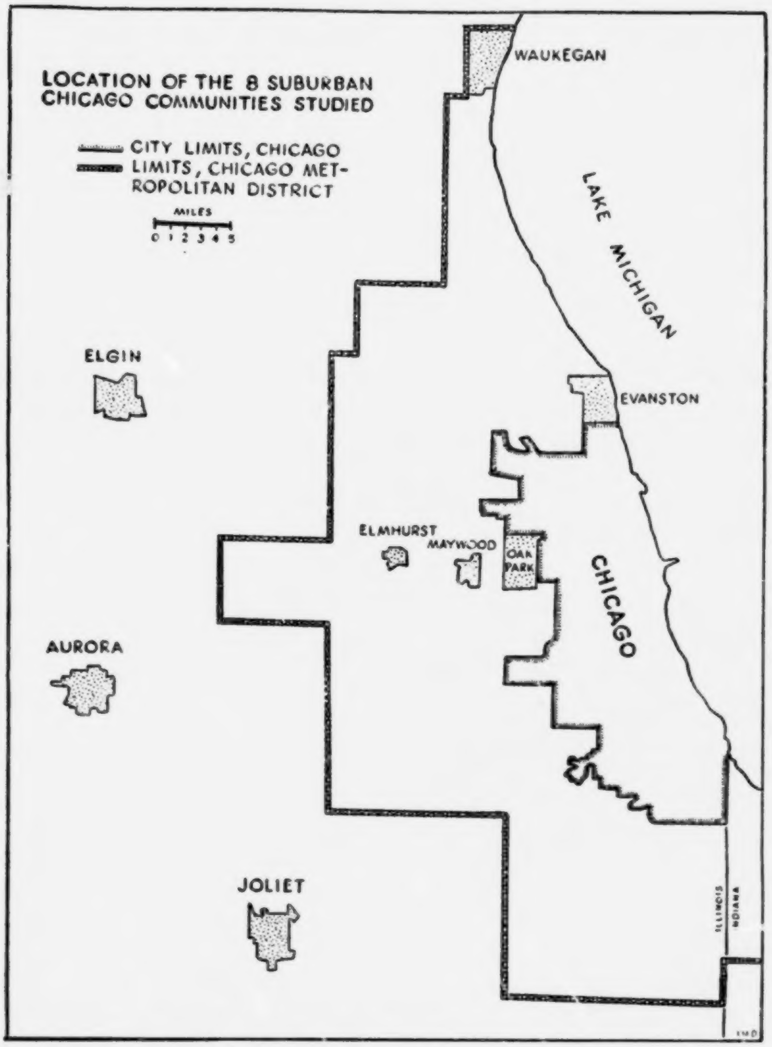


Figure 1.—*Location of the eight communities studied.*

0.9 part per million or more of fluoride (F) in their public water supplies. This work proved of value in selecting the areas to be studied.

The disclosure of a number of suburban Chicago communities with small amounts of fluorides in their domestic water supplies presented the unusual epidemiological opportunity of comparing their dental caries experience rates with those of their neighbors using the fluoride-free Lake Michigan water.

The communities selected for study, the findings of which form the basis of this report, were Elmhurst, Maywood, Aurora, Joliet, Elgin, Evanston, Oak Park, and Waukegan.

Their location with respect to the city of Chicago and the Chicago Metropolitan District is shown in figure 1.

Population of cities studied.—Population statistics with respect to the eight cities or villages studied are given in table 1. Inasmuch as the study was limited to white school children, the percentage of native white was computed on the basis of the total white population, not the total population. At Elmhurst, Maywood, Aurora, Joliet, Elgin, Evanston, Oak Park, and Waukegan the percentage of native white of the white population was 86.4, 84.0, 86.8, 82.8, 84.6, 83.5, 86.8, and 79.7 percent, respectively.

Climatological data.—Weather Bureau reports list the number of clear, partly cloudy, and cloudy days as recorded for a number of stations in northern Illinois. Reporting stations are located at three of the cities included in this study, viz. Aurora, Joliet, and Waukegan. No station is located at Oak Park but it is assumed that the recordings of the Cicero Station probably reflect this type of climatological data for Oak Park inasmuch as the corporate limits of Cicero and Oak Park adjoin. These same conditions might also be considered as probably applicable to Maywood, which is about 1½ miles west of Oak Park. The number of clear, partly cloudy, and cloudy days was not recorded at the Elgin Station and no stations are listed for Evanston and Elmhurst. Available data on this subject are shown in table 2.

Sampling method.—The group examined was selected in the following manner. The classroom or assembly hall was visited and the purposes of the survey explained to the teacher and the pupils. Those children who stated that they had lived in the city continuously since birth and had always used the common water supply for domestic purposes (drinking and cooking) were assembled in a separate group. This group was then further questioned to determine whether there had been any breaks in the continuity of their residence and water consumption. If questioning elicited information which disclosed breaks in the continuity of exposure (30 days in any calendar year excepted), the child was eliminated from further study. Those remaining constituted the group classified as continuously exposed since birth to the effects of the local water supply. Immediately after the selection of the group to be studied, the name, address, age, grade, continuity of residence, and other pertinent data were recorded on a sampling card, one being made out for each child. Each child was again carefully questioned several days later at the time of the clinical examination regarding his or her water history. This second cross-questioning at times revealed discontinuities in water history not brought out in the first questioning; the number further eliminated by this second questioning was in the neighborhood of 10 percent of the group for whom sampling cards had been made out previously.

All sampling was done by one individual (H. T. D.). With the exception of Evanston and the ninth grades (Freshman High School) at Oak Park, Maywood, Elmhurst, Joliet, Waukegan, and at the West Side High School at Aurora, each classroom or assembly hall was visited and the sampling done at that time. Excepting Aurora all communities included in this study have one large community high school and relatively large enrollments. To meet this particular condition a method of "home room" sampling was developed and carried out by each home room teacher, after instruction in the method to be followed. At Evanston all seventh, eighth, and ninth grade pupils attend one of three large schools. In this city it was necessary to utilize the home room method of sampling through the medium of the home room teacher for the entire group. Each of the three schools has a school physician, and the sampling was supervised by the school physician in collaboration with the one of us (H. T. D.) conducting the sampling aspects of the study.

This method of preliminary sampling by the home room teacher was necessary in 9 of the 67 schools included in the study, and the results obtained were quite satisfactory. All children selected by the home room teacher were subsequently questioned individually by the dental examiner when the water history was recorded prior to the clinical examination.

The samples examined generally represent all white public school children in the community with the requisites of continuity of exposure defined. All public schools in the community having a seventh, eighth, or ninth grade were included in the study, but no effort was made to locate 12–14-year-old children in grades other than those specified, with the exception of those instances where an appreciable number of children of the age group studied were in the sixth grade.

The percentage of continuous histories is unusually low at Evanston, Oak Park, and Maywood. This may be due to (*a*) the common practice of children of these three communities going away for summer vacations considerably in excess of 30 days, and (*b*) to the movement in and out of Chicago or between the highly urbanized communities adjacent to the corporate limits of Chicago.

Table 2.—*A 5-year summary of available data concerning number of clear, partly cloudy, and cloudy days recorded for cities included in this study, or from communities in their immediate vicinity*

[From Weather Bureau. Department of Agriculture]

Station	Number of days														
	Clear					Partly cloudy					Cloudy				
	1935	1936	1937	1938	1939	1935	1936	1937	1938	1939	1935	1936	1937	1938	1939
Aurora	175	179	182	176	199	54	92	74	53	59	136	95	109	136	107
Chicago (University)	85	101	114	112	132	98	92	109	90	105	182	143	142	163	128
Cicero	81	119	99	100	116	104	96	129	108	110	180	151	137	157	139
Joliet	135	174	159	166	167	75	83	87	74	80	155	109	119	125	118
Waukegan	146	153	164	205	195	122	145	155	75	82	97	68	46	85	88
Average, Northern Division (Illinois)	137	184	168	157	173	89	87	86	93	89	139	95	111	115	103

N. B.: A day is classified clear, partly cloudy, or cloudy, on the basis of hourly estimations, sunrise to sunset, as follows:

Clear	Sky averages $\frac{3}{10}$ or less obscured.
Partly cloudy	Sky averages $\frac{4}{10}$ to $\frac{7}{10}$, inclusive, obscured.
Cloudy	Sky averages more than $\frac{7}{10}$ obscured.

It might be well to digress for a moment and touch upon certain basic constants and pertinent interfering variables, particularly as they relate to the problem of sampling. As dental caries is a nonhealing lesion, a single clinical dental examination of 12–14-year-old children can merely record the amount of dental caries experienced by that group during the posteruptive life of the teeth examined. The observed lesion may have developed at any time during the posteruptive life of the tooth examined. At what particular time during the posteruptive tooth life the observed lesion developed cannot be determined on the basis of a single examination in this age group.

Table 1.—*Composition of the population of the 8 suburban Chicago communities studied (census of 1930)*

City	Total	White	Negro	Other races [1]	Total	White	Negro	Other races [1]	Native white of white population
	Number				Percent				
Elmhurst	14,055	14,023	13	19	100.0	99.77	0.09	0.14	86.4
Maywood	25,829	25,087	722	20	100.0	97.13	2.79	0.08	84.0
Aurora	46,589	45,348	936	305	100.0	97.34	2.01	0.65	86.8
Joliet	42,993	40,797	1,309	887	100.0	94.89	3.05	2.06	82.8
Elgin	35,929	35,539	310	80	100.0	98.92	0.86	0.22	84.6
Evanston	63,338	58,338	4,938	62	100.0	92.10	7.80	0.10	83.5
Oak Park	63,982	63,798	143	41	100.0	99.71	0.22	0.07	86.8
Waukegan	33,499	31,925	1,017	557	100.0	95.30	3.04	1.66	79.7

[1] While the Negro was excluded from this study because of the possibility of a racial difference in attack by dental caries, no attempt was made to eliminate children of "other races." This segment of the population was of such a relatively small percentage of the general population (0.6 percent) of the 8 communities studied that it seemed unnecessary to eliminate the occasional child who may have belonged in this classification. They are, accordingly, included with the white children in the tables that follow in this paper. Persons of Mexican birth or parentage who were not definitely reported as white or Indian were designated "Mexican" in the 1930 Census and included in the general class of "other races." In prior censuses most of the Mexicans have been classified as white. Of the 1,952 persons listed in this column, Elmhurst excluded, 1,755, or approximately 90 percent, were Mexicans.

The movement of populations, especially in the densely populated urbanized areas contiguous to the city of Chicago, is quite marked. The water supplies of these numerous communities show considerable variation, some purchasing Lake Michigan water from the city of Chicago, others depending wholly upon the municipal ground water supply. As single clinical examinations in this age group can disclose only the amount of dental caries experienced and not when it occurred, the corollary that naturally follows demands that all observations be confined to children with continuous exposure to the variable under study (the communal water supply). Otherwise, dental caries developed several years previously in an area with a high rate might be erroneously charged to an area with a low rate, or vice versa. In order, therefore, that differences in dental caries experience might be effectively studied with respect to their relationship to the mineral composition of the public water supply, all groups compared were placed on a comparable basis of exposure to risk, namely, age, sex, color, and continuous use of the water supply being studied.

Table 3 shows the number of public schools in which examinations were held, the number of 12–14-year-old pupils in attendance on the day of sampling, and the number and percentage of these whose histories on repeated questioning indicated continuity of exposure and who were examined. Attention might be called to the difference in the percentages of those with continuous histories between those communities contiguous to the city of Chicago (Evanston, Oak Park, and Maywood) and the more out-

Table 3.—*Summary of data with relation to continuity of exposure to the public water supply of 2,832 white 12–14-year-old children residing in 8 suburban Chicago communities*

Place	Number of public schools in which examinations were held	Number of 12–14-year-old children in attendance on day of sampling	Number of 12–14-year-old white children whose histories on repeated questioning [1] indicated continuity of exposure and who were examined	Percentage of the total present who were examined
Elmhurst	7	633	170	26.9
Maywood	6	873	171	19.6
Aurora	13	1,625	633	39.0
Joliet	6	1,412	447	31.7
Elgin	10	1,030	403	39.1
Evanston	3	2,125	[2] 256	12.0
Oak Park	12	1,662	329	19.8
Waukegan	10	1,354	423	31.2
Total	67	10,714	2,832	26.4

[1] About 20 percent of the group for whom sampling cards were originally made out were not examined. The detailed subsequent questioning which disclosed breaks in the continuity of exposure warranting elimination from the study accounted for about half of the cases excluded and these, together with those absent on the day of examination and the colored, comprised the 20 percent referred to.

[2] In addition there were 53 other children with a history of continuous exposure whose parents did not give their consent to making the clinical examination.

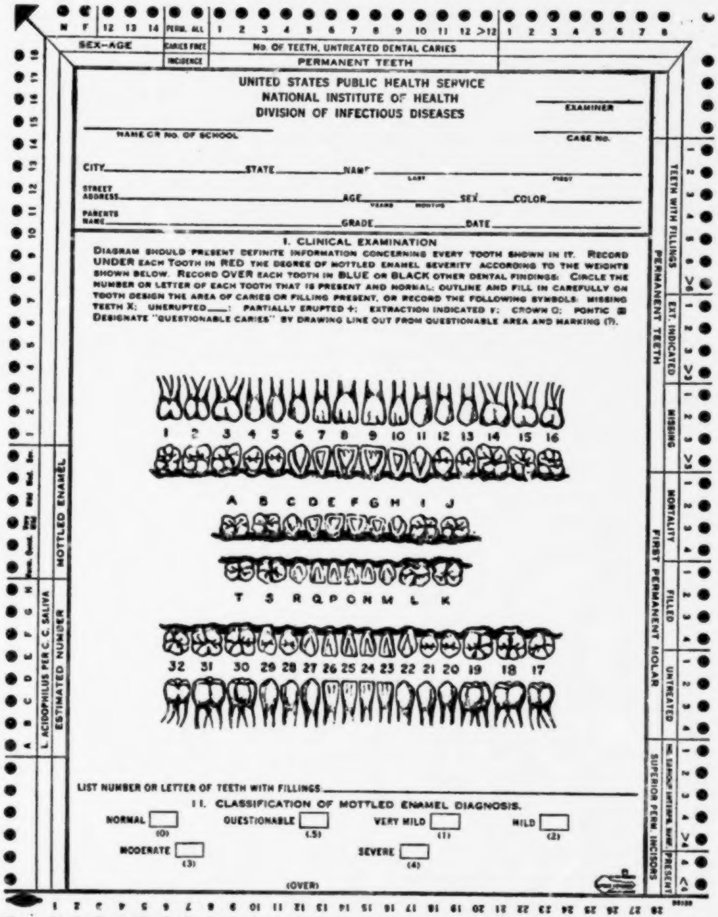
lying suburbs such as Waukegan, Elgin, Aurora, and Joliet.

Clinical examination.—All examinations were made by a dentist using a mouth mirror and explorer with the child seated facing a window. Explorers used throughout the study were double end No. 3. New explorers were provided at intervals throughout the study and Arkansas stones were furnished the examiners in order that the explorer points might be kept sharp at all times. In all instances the instruments used in the examinations were taken from the sterilizer and placed in a common pool from which the examiner selected the instrument to be used. Failure in coalescence of enamel lobes (pits and fissures) in which the end of the explorer caught but which showed no evidence of dental caries was not counted as caries. Pits or fissures showing one or more of the following criteria were counted as caries irrespective of how small the cavitation: Slight opacity around the edges, underlying dark stain suggestive of caries, or a perceptible soft feeling when the explorer was inserted in the pit or fissure. Examination of each child took approximately 10 minutes.

The personal interpretation in diagnosis is subject to some variation between examiners. This is especially noticeable in communities characterized by low dental caries experience rates where oftentimes pits and fissures introduce an important problem of subjective assessment. The inherent variation associated with subjective assessment results in varying degrees of differences between examiners in dental caries diagnosis. Attempts to equalize variation between the examiners (Assistant Dental Surgeons (R) David C. Johnston and Edwin M. Short), or at least to make such variations compensatory insofar as group differences in dental caries rates were concerned, were made as follows:

1. With respect to previous training both examiners had completed a year's postgraduate study in children's dentistry at the Forsyth Dental Infirmary, Boston, Mass., about a month prior to the beginning of this study. This 1 year of specialized training in the same class in a subject pertinent to this study, dental caries in children, should have a tendency to bring the examiners into closer diagnostic accord than if the study had been made by two examiners of dissimilar training.

2. At the beginning of the study, the diagnostic criteria of the two examiners were calibrated for several weeks by one


SEX–AGE | CARIES FREE | INCIDENCE | No. OF TEETH, UNTREATED DENTAL CARIES | PERMANENT TEETH

UNITED STATES PUBLIC HEALTH SERVICE
NATIONAL INSTITUTE OF HEALTH
DIVISION OF INFECTIOUS DISEASES

EXAMINER ____

NAME OR No. OF SCHOOL ____

CASE No. ____

CITY ____ STATE ____ NAME ____ LAST ____ FIRST

STREET ADDRESS ____ AGE ____ YEARS ____ MONTHS ____ SEX ____ COLOR ____

PARENTS NAME ____ GRADE ____ DATE ____

I. CLINICAL EXAMINATION

Diagram should present definite information concerning every tooth shown in it. Record UNDER each tooth in RED the degree of mottled enamel severity according to the weights shown below. Record OVER each tooth in BLUE or BLACK other dental findings: Circle the number or letter of each tooth that is present and normal; outline and fill in carefully on tooth design the area of caries or filling present, or record the following symbols: missing teeth X; unerupted ___; partially erupted +; extraction indicated ∨; crown O; pontic ⊠ Designate "questionable caries" by drawing line out from questionable area and marking (?).

LIST NUMBER OR LETTER OF TEETH WITH FILLINGS ____

II. CLASSIFICATION OF MOTTLED ENAMEL DIAGNOSIS.

NORMAL ☐ (0) QUESTIONABLE ☐ (.5) VERY MILD ☐ (1) MILD ☐ (2)

MODERATE ☐ (3) SEVERE ☐ (4)

(OVER)

Figure 2.—*Form used in dental caries study (front).*

of us (F. A. A., Jr.) in both an area with a high dental caries rate (Waukegan) and in one with a low dental caries rate (Maywood).

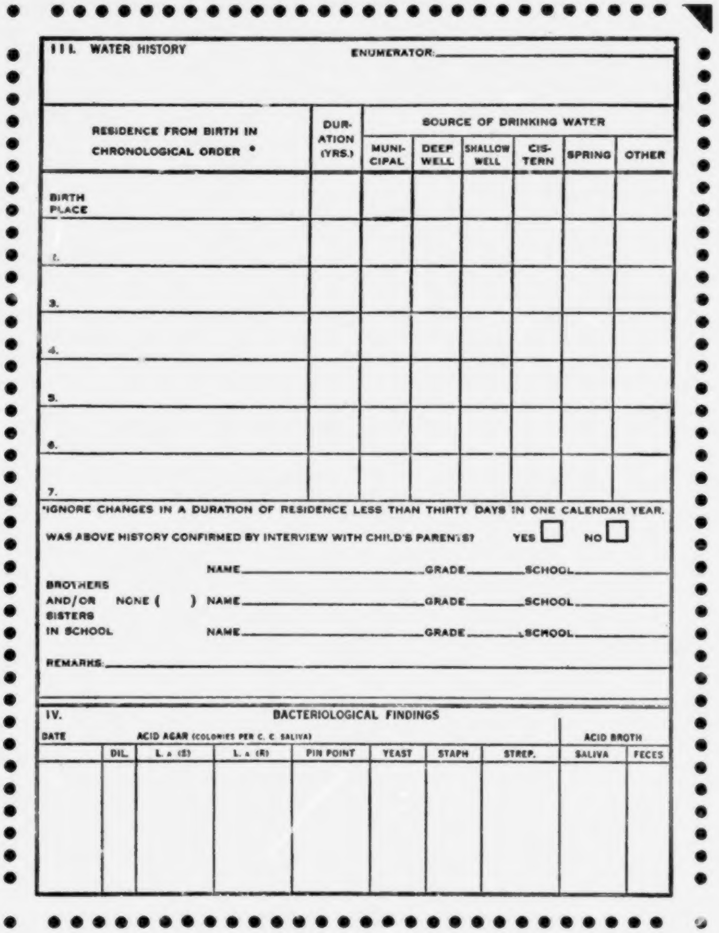
3. The examiners worked together as a team, visiting each school included in the study, and each examined approximately an equal number of children in each school. The examination schedules were numbered serially, and throughout the entire study all odd-numbered cases were examined by one examiner, all even-numbered by the other.

The clinical findings were recorded on a schedule form designed for combined dental caries and mottled enamel studies (fig. 2). The several communities were studied in this order: Waukegan, Maywood, Oak Park, Elmhurst, Elgin, Evanston, Aurora, and Joliet, the purpose being to have examiners alternate between areas which, on the basis of the fluoride content of the public water supply, might be expected to show high and low dental caries experience rates.

CLINICAL FINDINGS

In the eight communities studied, 2,832 children were examined; they were distributed according to age and sex as shown in table 4.

An analysis of the data in table 5 shows a remarkable difference in the amount of dental caries in these selected groups, both with respect to the percentage incidence of affection and to the dental caries experience. For instance, the combined dental caries experience rate for the 1,421 children of those communities (Elmhurst, Maywood, Aurora, and Joliet) whose public water supplies contain fluorides (F) in excess of 1.0 p.p.m. is 288 per 100 children in contrast to a rate of 746 per 100 children in the 1,008 children of communities (Evanston, Otk Park, and Waukegan) using water with a fluoride content of 0.0 p.p.m. In other words, there is 2.6 times as much dental caries in the latter communities as in the former.



III. WATER HISTORY

ENUMERATOR: ______

RESIDENCE FROM BIRTH IN CHRONOLOGICAL ORDER *	DURATION (YRS.)	SOURCE OF DRINKING WATER					
		MUNICIPAL	DEEP WELL	SHALLOW WELL	CISTERN	SPRING	OTHER
BIRTH PLACE							
2.							
3.							
4.							
5.							
6.							
7.							

*IGNORE CHANGES IN A DURATION OF RESIDENCE LESS THAN THIRTY DAYS IN ONE CALENDAR YEAR.

WAS ABOVE HISTORY CONFIRMED BY INTERVIEW WITH CHILD'S PARENTS? YES ☐ NO ☐

BROTHERS AND/OR SISTERS IN SCHOOL NONE ( )
NAME ______ GRADE ______ SCHOOL ______
NAME ______ GRADE ______ SCHOOL ______
NAME ______ GRADE ______ SCHOOL ______

REMARKS: ______

IV. BACTERIOLOGICAL FINDINGS

DATE	ACID AGAR (COLONIES PER C. C. SALIVA)							ACID BROTH	
	DIL.	L. a (S)	L. a (R)	PIN POINT	YEAST	STAPH	STREP.	SALIVA	FECES

FIGURE 2.—*Form used in dental caries study (back).*

With respect to the data on permanent teeth shown in table 5 it also seems desirable to list how much each of the following items contributed to the rates shown: Filled teeth (past dental caries), teeth with untreated dental caries, extraction indicated, and missing teeth (teeth lost because of accident, or extracted because of malposition excluded). These data are shown in table 6 and figure 3.

Proximal dental caries.—An unusual difference in the amount of dental caries in the proximal surfaces of the four superior permanent incisors was noted in the four Illinois cities previously studied (*2*). At Macomb and Quincy there was about 16 times as much of this type of dental caries as was observed in the children of Galesburg and Monmouth.

The dental caries experience of the eight proximal surfaces of the four superior permanent incisors in the children of the eight communities included in this report is shown in table 7.

Differences of approximately the same order of magnitude as previously reported were found in the communities included in this study. For example, in the cities using a fluoride-free water (Evanston, Oak Park, and Waukegan) dental caries experience was evidenced in 1,043 out of 7,968 surfaces, or a rate of 13.1 per 100 surfaces. In the 11,256 proximal surfaces in the children exposed to waters with a fluoride (F) content in excess of 1.0 p.p.m. (Elmhurst, Maywood, Aurora, and Joliet) evidence of dental caries was discernible in only 103, or a rate of 0.9 per 100 surfaces. Or, to summarize, there was 14.3 times as much of this type of caries in the former group as in the latter group.

First permanent molar mortality.—An index of value for measuring certain aspects of the dental caries problem is the first permanent molar mortality rate. Knutson and Klein (*4*) define tooth mortality as referring to "not only extracted permanent teeth but also those which are indicated for extraction and still present in the mouth"; molar mortality rates reported in table 8 were computed in accordance with this definition. In order to determine how closely this index might reflect differences in the dental caries experience of the eight surveyed communities, the first permanent molar mortality rate for each community was computed. Furthermore, as tooth mortality may be influenced by the amount of remedial treatment received, data with respect to the number and percent of filled per-

TABLE 4.—*Distribution of the 2,832 children examined, according to age and sex*

City	Total	Age in years, last birthday								
		12			13			14		
		M	F	Both sexes	M	F	Both sexes	M	F	Both sexes
	Number									
Elmhurst	170	36	28	64	31	29	60	22	24	46
Maywood	171	34	20	54	31	33	64	27	26	53
Aurora	633	120	88	208	121	104	225	105	95	200
Joliet	447	67	60	127	66	90	156	63	101	164
Elgin	403	72	90	162	52	55	107	75	59	134
Evanston	256	30	52	82	48	54	102	33	39	72
Oak Park	329	55	66	121	49	54	103	54	51	105
Waukegan	423	60	70	130	62	90	152	68	73	141
	Percent									
Elmhurst	100.0	21.2	16.5	37.7	18.2	17.1	35.3	12.9	14.1	27.0
Maywood	100.0	19.9	11.7	31.6	18.1	19.3	37.4	15.8	15.2	31.0
Aurora	100.0	19.0	13.9	32.9	19.1	16.4	35.5	16.6	15.0	31.6
Joliet	100.0	15.0	13.4	28.4	14.8	20.1	34.9	14.1	22.6	36.7
Elgin	100.0	17.9	22.3	40.2	12.9	13.7	26.6	18.6	14.6	33.2
Evanston	100.0	11.7	20.3	32.0	18.8	21.1	39.9	12.9	15.2	28.1
Oak Park	100.0	16.7	20.1	36.8	14.9	16.4	31.3	16.4	15.5	31.9
Waukegan	100.0	14.2	16.6	30.8	14.6	21.3	35.9	16.1	17.2	33.3

manent molars are furnished for a fuller interpretation of the molar mortality rates reported.

Incidence of endemic dental fluorosis (mottled enamel).—The incidence and degree of mottled enamel observed in the groups studied is shown in table 9.

In accordance with a previously described method of computing a community mottled enamel index (*5*) on the basis of the percentage distribution of clinical severity, the approximate mottled enamel index of Elmhurst is "slight"; that of Maywood, Aurora, and Joliet, "border-line"; and that of Elgin, Evanston, Oak Park, and Waukegan, "negative."

Graduations in the amount of dental caries.—A study of the differences in the amount of dental caries between, for instance, Aurora, whose public water supply contains 1.2 p.p.m. of fluoride (F), and the communities using fluoride-free water (Evanston, Oak Park, and Waukegan) indicates that the vigor or force of the dental caries impact upon the selected populations studied varies widely. The 633 Aurora children showed a dental caries experience rate (permanent teeth) of 281 per 100 children; the 1,008 children at Evanston, Oak Park, and Waukegan, a rate of 746 per 100 children. The differences in the intensity of dental caries attack in Aurora and in the three other communities are shown in table 10 and figure 4.

Bacteriological studies.—In order to learn whether or not group differences in oral lactobacilli counts existed in the eight communities studied, *L. acidophilus* counts were made on saliva samples from 1,761 of the 2,832 children examined. In each community a specimen of saliva was collected from a representative sample of children selected at random from the total of those who were examined clinically. The bacteriological studies were conducted by two of the authors (P. J. and F. A. A. Jr.).

All saliva samples were collected in a similar manner at the same time of day (between 10 a.m. and 11:30 a.m.). Paraffin was used to stimulate the flow of saliva; all children were instructed to chew in such a manner as to touch all the teeth in the mouth. The time required to collect the saliva was approximately 5 minutes in all cases. The dilution used for all specimens was 1 cc. of saliva to 4 cc. of broth; 0.1 cc. of this mixture was plated on tomato juice agar of pH 5. Plates were incubated for 4 days and the counts of the *L. acidophilus* colonies were made by one of the authors (P. J.).

For a more detailed analysis of the relationship between the clinical findings and the bacteriological results, a separate tabulation was made of the dental caries experience of those children included in the bacteriological studies. It is shown in table 11.

The quantitative distribution of the lactobacilli counts are shown in table 12.

In order to demonstrate graphically the quantitative distribution of the *L. acidophilus* counts the entire group was divided into three classes: Those children using a water supply containing more than 1 p.p.m. F (Elmhurst, Maywood, Aurora, and Joliet); children whose water supply contained 0.5 p.p.m. F (Elgin); and children using water free of fluorine (Evanston, Oak Park, and Waukegan). This graphic presentation is shown in figure 5.

PUBLIC WATER SUPPLIES

Description of public water supplies.—For a proper evaluation of the reported

TABLE 6.—*Summary of the dental caries experience in the permanent teeth of 2,832 white school children, aged 12–14 years, of 8 suburban Chicago communities classified on the basis of filled teeth (past dental caries), untreated dental caries, extraction indicated, and missing teeth (presumably because of dental caries)*

City	Number of children examined	Dental caries experience, permanent teeth				
		Filled teeth (past dental caries) (a)	Teeth with untreated dental caries (b)	Extraction indicated (c)	Missing teeth (d)	Total (a+b+c+d)
		(A) Number				
Elmhurst	170	234	173	12	10	429
Maywood	171	216	202	3	21	442
Aurora	633	629	1,055	22	72	1,778
Joliet	447	468	879	18	78	1,443
Elgin	403	781	918	24	66	1,789
Evanston	256	985	614	24	100	1,723
Oak Park	329	1,546	715	18	95	2,374
Waukegan	423	1,527	1,536	70	294	3,427
		(B) Number per 100 children				
Elmhurst		137.6	101.8	7.1	5.9	252
Maywood		126.3	118.1	1.8	12.3	258
Aurora		99.4	166.7	3.5	11.4	281
Joliet		104.7	196.6	4.0	17.4	323
Elgin		193.8	227.8	6.0	16.4	444
Evanston		384.8	239.8	9.4	39.1	673
Oak Park		469.9	217.3	5.5	28.9	722
Waukegan		361.0	363.1	16.5	69.5	810

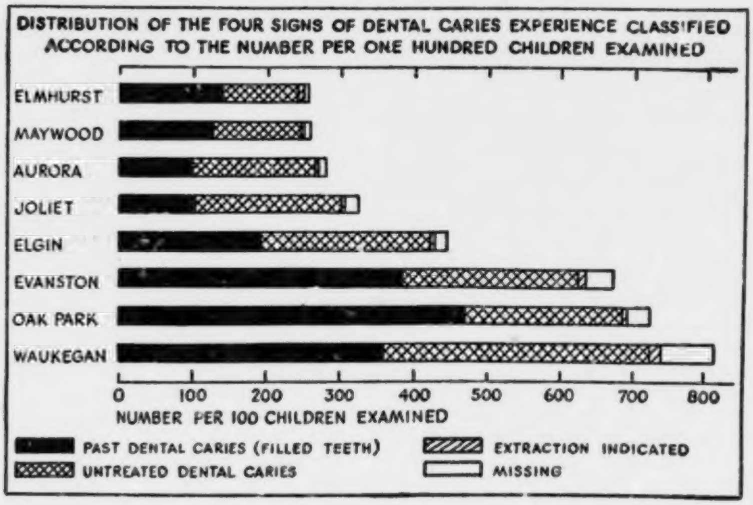


FIGURE 3

TABLE 7.—*Dental caries experience of the proximal surfaces of the four superior permanent incisors of selected children of 8 suburban Chicago communities*

City	Number of children examined	Number of proximal surfaces [1]	Number of proximal surfaces with dental caries experience	Dental caries experience per 100 surfaces
Elmhurst	170	1,342	8	0.60
Maywood	171	1,358	8	.59
Aurora	633	5,000	39	.78
Joliet	447	3,556	48	1.3
Elgin	403	3,170	130	4.1
Evanston	256	2,030	218	10.7
Oak Park	329	2,584	232	9.0
Waukegan	423	3,354	593	17.7

[1] Teeth lost by accident, unerupted, extracted because of malposition, and proximal surfaces restored by prosthesis (inlays, ¾ crowns, etc.) because of traumatic injury, excluded. The maximum possible number of surfaces in a population of this size (2,832) is 22,656. The number of surfaces excluded for the reasons stated was 262, or approximately 1.2 percent.

TABLE 8.—*Summary of data respecting first permanent molar mortality rates, including number and percent of filled teeth, in selected 12–14-year-old children of 8 suburban Chicago communities*

[All teeth referred to in this table are first permanent molars]

City or village	Elmhurst	Maywood	Aurora	Joliet	Elgin	Evanston	Oak Park	Waukegan
Number of children examined	170	171	633	447	403	256	329	423
Molar population—estimated (number of children examined × 4)	680	684	2,532	1,788	1,612	1,024	1,316	1,692
Percent of children with 1 or more missing teeth (extraction indicated included)	7.6	8.2	10.4	12.8	13.9	23.4	17.9	40.9
Number of teeth showing dental caries experience:								
(a) Filled teeth	197	159	464	339	559	593	883	724
(b) Untreated dental caries	88	99	533	441	428	164	148	372
(c+d) Extraction indicated and missing	20	20	92	87	82	109	102	338
(a+b+c+d) Total	305	278	1,089	867	1,069	866	1,133	1,434
Percent of teeth showing dental caries experience	44.9	40.6	43.0	48.5	66.3	84.6	86.1	84.8
First permanent molar mortality, number per 100 children	11.8	11.7	14.5	19.5	20.3	42.6	31.0	79.9
Percent of teeth with dental caries experience, that are filled $\frac{a}{a+b+c+d}$	64.6	57.2	42.6	39.1	52.3	68.5	77.9	50.5

clinical findings and their relation to the fluorine-dental caries hypothesis, detailed data regarding the common water supplies are included for the period of time concomitant with the life of the group examined. Description and data concerning these municipal water supplies were obtained from Mr. C. W. Klassen, chief sanitary engineer, State Department of Public Health, from the District Sanitary Engineers in whose districts the cities are located, from Bulletin No. 21, including Supplement No. 1 thereto, of the State Water Survey Division, and by interview with the local water superintendent. Description of these supplies follows.

Elmhurst.—The public water supply of Elmhurst is obtained from four drilled wells. Wells Nos. 1, 2, and 3 are located in the north central part of the city, No. 4 near the western city limits.

Well No. 1 was drilled in 1916 to a depth of 957 feet. Well No. 2 is located about 100 feet from the well No. 1 and was drilled in 1919 to a depth of 1,398 feet. It enters the Eau Claire formation of the Cambrian system. In 1926–27 well No. 2 was deepened to 2,222 feet.

Well No. 3 is located about two blocks from wells Nos. 1 and 2 and was drilled in 1925–26 to a depth of 2,077 feet, this level being designated in the log as the Mount Simon sandstone. In 1933 this well was deepened to 2,221 feet.

Well No. 4 was drilled in 1928, originally to a depth of 2,205 feet into the Mount Simon formation. In 1937 the lower part of this well was plugged in order to reduce the high sodium chloride content and the water is now being obtained from a depth of about 1,450 feet which is in the Eau Claire formation. Well No. 4 is used only sporadically, generally during the summer months.

At the time of the survey well No. 1 was emptying into a 150,000 gallon reservoir which has two leads, one to a large 1,000,000 gallon reservoir, the other direct to the mains. Well No. 1 is generally pumped between midnight and 6 a.m.; the water from this well is pumped directly into the mains during this period. Wells Nos. 2 and 3 supply most of the water used by the city between 6 a.m. and midnight. A sample of water from each of these two wells, collected in December 1939, showed a fluoride (F) content of 1.3 and 2.2 parts per million, respectively. Another sample collected in September 1939, from a tap located at the City Hall showed a fluoride content of 1.8 parts per million.

Maywood.—The present Maywood supply is obtained from four drilled wells, designated at wells Nos. 3, 4, 5, and 6. Wells Nos. 1 and 2 were drilled about 1895; well No. 1 was abandoned about 1919, No. 2 about 1923.

Well No. 3 was drilled in 1910 and is

1,800 feet deep. Between 840 feet and 980 feet the St. Peter sandstone was penetrated and Cambrian sandstone entered at a depth of 1,400 feet. This well was repaired in 1931, the repair including the placing of 528 feet of 12-inch casing cemented in place for its entire length, and the reaming of the balance of the hole to 10 inches. Well No. 4 was completed in 1918 and is 2,048 feet deep. St. Peter sandstone was entered at a depth of 897 feet and the Cambrian sandstone at 1,400 feet. The well is cased 612 feet, about 50 feet into the Galena-Plattsville limestone. In 1937 the lower 30 feet was filled with cement. The discharges from wells Nos. 3 and 4 are softened (zeolite) and collected in the "north" reservoir.

Well No. 5 was drilled in 1922 and is 2,076 feet deep. This well is 17 inches in diameter at the top and is cased to a depth of 545 feet. A 10-inch liner is placed between depths 1,040 feet and 1,100 feet. This well is 10 inches in diameter at the bottom where it enters the Mount Simon of the Cambrian system. Apparently no changes have been made in this well since its original installation except for a cleaning out in 1938. Well No. 6 was drilled in 1924 to an original depth of 2,090 feet but in 1937 this was filled back to a depth of 1,549 feet. The bottom of the original hole was filled with 40 feet of concrete, and after shooting the well at 1,440 to 1,470 feet about 455 feet of sand and clay was allowed to fill the well. Then more cement was added to seal the lower part of the well. The chloride content was reduced by this procedure from 1,500 to 250 parts per million. This well is cased to a depth of 556 feet. Wells Nos. 5 and 6 are located about a mile and a half from wells Nos. 3 and 4. The discharges from wells Nos. 5 and 6 are softened (zeolite) and collected in the "south" reservoir. The gravity open-type softening plant and chlorination was installed in 1938.

TABLE 9.—*Incidence and distribution of endemic dental fluorosis (mottled enamel) according to the degree of affection*

Macroscopic signs of mottled enamel	Elmhurst	Maywood	Aurora	Joliet	Elgin	Evanston	Oak Park	Waukegan
	(A) Number							
Total examined	170	171	633	447	403	256	329	423
Absent:								
Normal	48	67	337	181	244	235	298	414
Questionable	54	47	201	153	142	17	29	8
Present:								
Very mild	51	50	88	99	14	4	2	1
Mild	15	7	7	14	3	0	0	0
Moderate	2	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0
	(B) Percent							
Total examined	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Absent:								
Normal	28.2	39.2	53.2	40.5	60.5	91.8	90.6	97.9
Questionable	31.8	27.5	31.8	34.2	35.3	6.6	8.8	1.9
Present:								
Very mild	30.0	29.2	13.9	22.2	3.5	1.6	.6	.2
Mild	8.8	4.1	1.1	3.1	.7	0	0	0
Moderate	1.2	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0
Incidence of affection	40.0	33.3	15.0	25.3	4.2	1.6	.6	.2

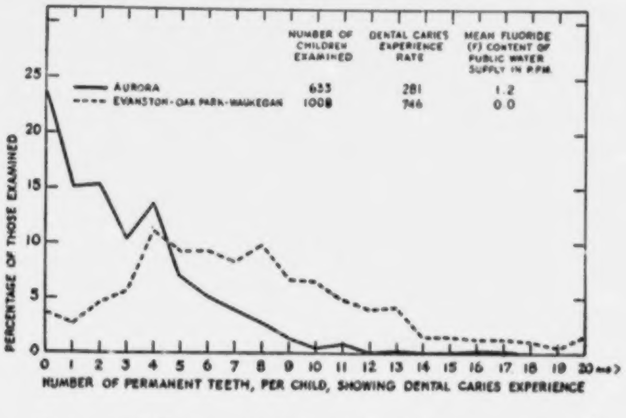


FIGURE 4.—*Differences in the severity of dental caries attack.*

Aurora.—The common water supply of Aurora is obtained from nine wells, four of which (Nos. 5, 11, 12, and 12A) pump into a collecting reservoir and thence to the distribution system. This reservoir and well field is located just outside the city limits, north of the city. The remaining wells (Nos. 6, 7, 8, 9, and 10) are located in different sections of the city. All pump directly into the distribution system with the exception of well No. 10, which pumps to the distribution system through a sand-collecting reservoir. Although there are several points in the distribution system where the water from several wells mix, these points are very indefinite as wells are not operated at the same time or for the same length of time. From the pumping records of the Water Department, wells Nos. 11, 12, and 12A (distributed from the common reservoir) have furnished during 1935, 1936, 1937, 1938, and the first 9 months of 1939, 56, 49, 68, 70, and 61 percent, respectively, of the water pumped (no water was pumped from the recently repaired well No. 5 during this period). Water from the main pumping station (wells Nos. 11, 12, and 12A) passes to the center of the city through main pipe lines lying east and west of the Fox River. Water from this source reaches all parts of the city, although in certain sections there is some mixture of waters from those wells pumping directly into the distribution system. The location of the wells, the reservoir, and the main pipe lines are shown in figure 8.

Wells Nos. 1 to 4, inclusive, drilled between 1891 and 1895, are now abandoned and plugged. Their site is close to the present location of wells Nos. 5, 11, 12, and 12A. Data with respect to these wells follow. (See p. 117.)

In the preliminary studies, samples were collected from the common reservoir (wells Nos. 11, 12, and 12A) and from each of the wells Nos. 6 to 10, inclusive. The monthly water samples for a year were collected from the main pumping station (wells Nos. 11, 12, and 12A). On the basis of the 12 months' pumping records for 1938, the percentages of water pumped were calculated and are shown with the fluoride (F) con-

Well No.	Year drilled	Depth in feet	Remarks
1	1891	1,388	Deepened in 1898 to 2,230 feet. A report in city water department gives the depth as 2,250 feet.
2	1892	2,230	
3	1893	2,230	
4	1895	2,230	

The record with respect to the wells now in use follows:

Well No.	Year drilled	Depth in feet	Well No.	Year drilled	Depth in feet
5	1910	2,250	10	1923	2,290
6	1915	2,185	11	1928	2,253
7	1916	2,250	12	1929	2,260
8	1916	2,280	12A	1936	2,250
9	1923	2,259			

Well No.	Percent contributed by each well and main group of wells to the total amount of water used in Aurora during 1938	Fluoride (F) content in p. p. m. of samples collected in August 1939
11, 12, and 12A	70	1.2
10	1	1.1
9	7	1.3
8	8	1.3
7	9	.7
6	5	.5

tent of each supply in the table immediately above.

The Aurora water supply is not treated except for provisions for emergency chlorination.

From the standpoint of a population exposed for a long period of time to a public water supply containing small amounts of fluorides, Aurora appears to offer many advantages for epidemiological study. Since 1898 the public water supply has been obtained from wells into the Cambrian "Potsdam" sandstone.

TABLE 10.—*Differences in the severity of dental caries attack in selected school children at Aurora (Ill.), whose public water supply contains 1.2 p.p.m. of fluoride (F), and three communities (Evanston, Oak Park, and Waukegan) using fluoride-free water*

Number of permanent teeth showing dental caries experience in amounts as specified per child	Aurora			Evanston, Oak Park, Waukegan		
	Number of children	Percentage of total examined	Percentage of total (cumulative)	Number of children	Percentage of total examined	Percentage of total (cumulative)
0	149	23.5	23.5	37	3.7	3.7
1	95	15.0	38.5	27	2.7	6.4
2	97	15.3	53.8	46	4.6	11.0
3	66	10.4	64.2	57	5.6	16.6
4	87	13.7	77.9	113	11.2	27.8
5	44	7.0	84.9	94	9.3	37.1
6	33	5.2	90.1	95	9.4	46.5
7	25	3.9	94.0	84	8.3	54.8
8	17	2.7	96.7	101	10.0	64.8
9	8	1.3	98.0	68	6.7	71.5
10	3	.5	98.5	67	6.6	78.1
11	6	.9	99.4	49	4.9	83.0
12	0			40	4.0	87.0
13	1	.2	99.6	42	4.2	91.2
14	0			15	1.5	92.7
15	0			15	1.5	94.2
16	1	.2	99.8	13	1.3	95.5
17	1	.2	100.0	13	1.3	96.8
18				11	1.1	97.9
19				6	.6	98.5
20 and over				15	1.5	100.0
Total	633			1,008		

Dental caries experience, number per 100 children examined: Aurora—281; Evanston, Oak Park, Waukegan—746.

Joliet.—At the time of the survey the Joliet water supply was being obtained from three wells, Washington Street wells Nos. 1 and 2, and from the Ottawa Street well. The water supply of this city has undergone a number of physical changes during the past 12 to 14 years, but an analysis of such information as is available does not indicate any marked change in the fluoride (F) content of the water used by the inhabitants. During this period the water supply of Joliet was largely obtained from wells into the Cambrian sandstone, and aquifer whose waters often contain small amounts of fluorides. Weart and Klassen (5) in 1937 reported, on the basis of seven samples collected in 1936, that the fluoride content of this public water supply was 1.4 p.p.m. The samples collected during the past year show a mean fluoride content of 1.3 p.p.m. The degree of mottled enamel observed in the 12–14-year-old children examined in this survey would reflect the fluoride concentration of the public water in use roughly 9 to 12 years ago. The percentage incidence of affection observed points to a concentration in the neighborhood of 1.3 to 1.4 p.p.m. Hence, with the exception of the summer of 1930, which will be discussed later, both chemical and epidemiological evidence would suggest that the fluoride (F) content of the water used in this community did not differ greatly from the range of concentration stated.

The following wells have contributed to the Joliet water supply:

Name of well	Depth in feet	Year drilled	Year last in use
Washington St.:			
Well No. 1	1,608	1937	(1)
Well No. 2	1,704	1900	(1)
Ottawa St.	1,627	1907	(1)
Canal St.	1,570	1911	1931
Spruce Slip	1,530	1912	1931
Van Buren St.	1,550	1913	1935
Des Plaines St.	1,560	1913	1937
Ruby St.	1,564	1915	1937
Jasper St.	1,565	1930	1938
Williamson St.	1,588	1925	1938

[1] In use at present.

Washington Street well No. 2 and the Ottawa Street well were repaired in 1937. Water from Washington Street wells Nos. 1 and 2 pass through a common collecting reservoir. During the period 1925–39, certain wells now not in use were being pumped directly into the distribution system.

In May 1930, because of a breakdown in the Williamson Avenue (Charlesworth Avenue) well, the public water supply was supplemented by water pumped from a quarry known as "Michigan Beach" and located on the south edge

TABLE 11.—*Summary of the percentage incidence and dental caries experience, permanent teeth, in the 1,761 children for whom a single L. acidophilus count was made*

City	Number of children examined	Children showing dental caries experience	Children showing no dental caries experience	Dental caries experience, permanent teeth: Filled teeth (past dental caries) (a)	Teeth with untreated dental caries (b)	Extraction indicated (c)	Missing (d)	Total (a+b+c+d)
				(A) Number				
Elmhurst	154	112	42	220	147	7	7	381
Maywood	139	100	39	174	164	1	13	352
Aurora	340	255	85	360	556	7	34	957
Joliet	233	191	42	265	469	10	41	785
Elgin	250	223	27	529	336	7	41	1,113
Evanston	208	200	8	802	485	23	89	1,399
Oak Park	208	202	6	1,010	424	13	61	1,508
Waukegan	229	223	6	884	816	31	160	1,891
	Percent of total[1] examined clinically			(B) Number per 100 children				
Elmhurst	90.6	72.7	27.3	143	95	4.5	4.5	247
Maywood	81.3	71.9	28.1	125	118	.7	9.4	253
Aurora	53.7	75.0	25.0	106	164	2.1	10.0	281
Joliet	52.1	82.0	18.0	114	201	4.3	17.6	337
Elgin	62.0	89.2	10.8	212	214	2.8	16.4	445
Evanston	81.3	96.2	3.8	386	233	11.1	42.8	673
Oak Park	63.2	97.1	2.9	486	204	6.3	29.3	725
Waukegan	54.1	97.4	2.6	386	356	13.5	69.9	826

[1] Percentage of total children examined clinically (tables 5 and 6) who were examined bacteriologically.

of the city. Up to the time of its use for drinking water it had been used for swimming-pool water. The quarry was of unknown depth and presumably was fed by springs. This water was chlorinated before being turned into the distribution system. According to the records of the State Department of Public Health about 1,000,000 gallons of water per day were used from the quarry and this continued for several months. The quarry water contributed about 25 percent of the water used by the inhabitants during this period. There is also a record of certain shallow wells, now abandoned, having constituted part of the city water supply.

At present the Washington Street wells supply a little over half of the water used in the city; the Ottawa Street well supplies the remainder. In the preliminary studies a sample of water from a tap in the Woodruff Hotel (southeastern section of the business district where water usage is high and located so that mixing of the two water supplies is obtained) showed on analysis a fluoride (F) content of 1.2 parts per million.

The present Joliet water supply is chlorinated.

Elgin.—Since 1905 Elgin as obtained its public water supply from ground water sources, previously from the Fox River. At present practically all of the water is obtained from six wells. Four of these wells are at the north end of the city (Main Station, Slade Avenue) and two in the southeastern section of the city (Lavoie Avenue well and the St. Charles Street well). In addition, the Creighton Avenue well and the Schuler Street well supply varying amounts, pumping directly into the distribution system.

Water from the four north wells passes through a softening plant to a reservoir and thence to the distribution system. In the southeastern part of town there is an aerator and settling reservoir at the site of the St. Charles Street well and the water from the Lavoie Avenue well, in addition to water from the St. Charles Street well, is regularly pumped over and through this aerator and reservoir before passing into the distribution system.

The major portion of the supply has been obtained for a number of years from the four north wells. For the years 1932, 1933, 1934, 1935, 1936, 1937, 1938, and 1939 the pumping records of the Water Department show that these four wells supplied 84, 78, 72, 71, 69, 65, 62, and 64 (estimated) percent, respectively, of the total amount of the water pumped.

When first installed these four wells ranged in depth from 1,300 to 2,000 feet. Between 1905 and 1917 certain changes were made by filling in the lower parts of the wells. In 1926 the wells were deepened and cleaned out to a depth of 1,960 feet and cased to a depth of about 122 feet. The four wells penetrate the Cambrian sandstone.

The southeastern section of the city is largely supplied with water from the Lavoie Avenue well and the St. Charles Street well. The former was drilled in 1931 to a depth of 675 feet and since 1933 has accounted for an increasing percentage of the water used. The St. Charles Street well is 100 feet deep and was drilled in 1933, replacing one at the same location drilled in 1921 to a depth of 101 feet. During July 1940, the St. Charles Street well furnished nearly 5 percent of the total pumpage from all wells, in August, about 1.8 percent.

The Schuler Street well, 1,960 feet deep and installed in 1932, is pumped irregularly, being used largely for standby purposes. This well and the nearby Creighton Avenue well pump directly into the distribution system. The Creighton Avenue well is 48 feet deep. A sample from this well collected in December 1939 showed a fluoride (F) content of 0.5 p.p.m. The date of its installation is not known, but it was reported in operation as early as 1928. During April, May, June, and July 1940, the Creighton Avenue well furnished 4.7, 9.6, 4.1, and 1.2 percent, respectively, of the total pumpage from all wells.

The two shallow wells (Creighton Avenue and St. Charles Street) supplied between 1934 and 1938 as much as 10 to 25 percent of the water pumped.

As has been noted, there is no common reservoir to which the water is pumped prior to turning it into the distribution system, and the southeastern section in all probability used a water differing in mineral composition from that used in the rest of the city. In the preliminary studies, samples were collected of the treated water at the Main Station (Slade Avenue), another at the City Hall, which is about the center of the mixed area, and should represent a sample of the mixture of the water from both ends of town, and a third sample from the reservoir served by the two wells at the south end of the city. These samples showed a fluoride (F) content of 0.4, 0.4, and 1.0 part per million, respectively. In 1938 a water softening plant using the lime process was installed for the treatment of the public water supply. About 15.6 grains of lime and ½ grain of alum per gallon are applied, followed by chlorination.

In order to learn whether the softening treatment was materially influencing the fluoride content of the water from the four north wells (Slade Avenue Station) raw (untreated) water samples were collected during November 1940 from wells

Table 12.—*Summary of the percentage distribution of oral L. acidophilus in salivas from 1,761 school children in 8 suburban Chicago communities*

City	Distribution of children according to the number of *L. acidophilus* per cc. of saliva								
	Negative	Less than 100	100 to 1,000	1,000 to 3,000	3,000 to 12,000	12,000 to 21,000	21,000 to 30,000	30,000 and over	Total
	(A) Number								
Elmhurst	39	16	13	9	16	13	10	38	154
Maywood	35	20	14	10	17	8	6	29	139
Aurora	86	44	24	10	43	34	12	87	340
Joliet	61	22	20	6	24	26	12	62	233
Elgin	40	13	24	14	40	22	14	83	250
Evanston	19	13	19	2	28	29	11	87	208
Oak Park	24	14	13	14	27	19	6	91	208
Waukegan	21	13	13	6	27	15	9	125	229
	(B) Percent								
Elmhurst	25.3	10.4	8.4	5.9	10.4	8.4	6.5	24.7	100
Maywood	25.2	14.4	10.1	7.2	12.2	5.7	4.3	20.9	100
Aurora	25.3	12.9	7.1	2.9	12.7	10.0	3.5	25.6	100
Joliet	26.2	9.4	8.6	2.6	10.3	11.2	5.1	26.6	100
Elgin	16.0	5.2	9.6	5.6	16.0	8.8	5.6	33.2	100
Evanston	9.1	6.3	9.1	1.0	13.5	13.9	5.3	41.8	100
Oak Park	11.5	6.7	6.3	6.7	13.0	9.1	2.9	43.8	100
Waukegan	9.2	5.7	5.7	2.6	11.8	6.5	3.9	54.6	100

Nos. 1, 2, 3, and 4; the fluoride (F) content of these samples was 0.7, 0.6, 0.5, and 0.5 part per million, respectively. Klassen states that tests made in 1936 of two samples from the Slade Avenue Station showed a fluoride content of 0.7 and 0.5 part per million. A sample at the same time from the St. Charles Street well showed a fluoride content of 1.2 parts per million.

Evanston.—The supply is obtained from Lake Michigan and is treated in a modern water purification plant by mixing, settling, rapid sand filtration, and chlorination. The mixing period is about 17 minutes, followed by a 2-hour settling period. There are 12 rapid sand filters, each having a capacity of 1,400 gallons per minute. About 0.7 grains per gallon of alum is applied for coagulation purposes and 0.3 parts per million of chlorine for sterilization. From 1874 to 1911, when chlorination by the use of hypochlorite of lime was started, the supply was obtained direct from Lake Michigan

PERCENTAGE DISTRIBUTION OF LACTOBACILLI IN THE SALIVA OF 1,761 CHILDREN EXAMINED IN EIGHT SUBURBAN CHICAGO COMMUNITIES GROUPED ACCORDING TO THE FLUORIDE (F) CONTENT OF THE PUBLIC WATER SUPPLY AND CLASSIFIED ACCORDING TO THE ESTIMATED NUMBER OF LACTOBACILLI PER CC. OF SALIVA

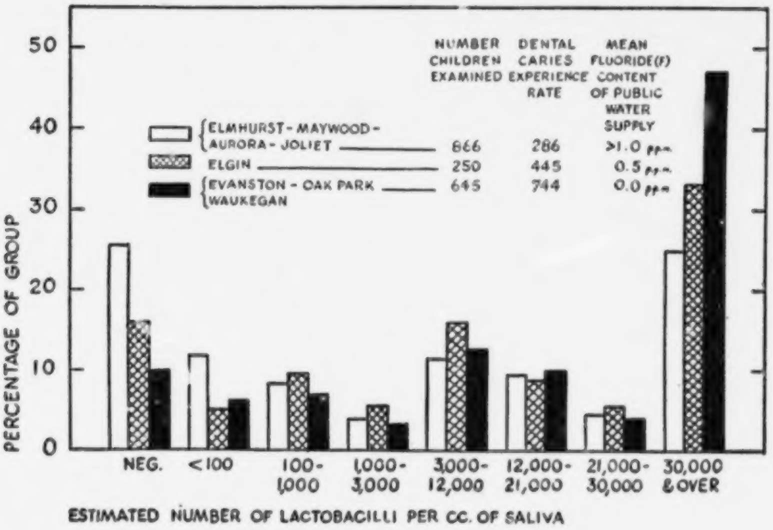


Figure 5

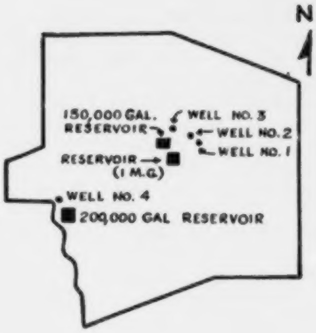
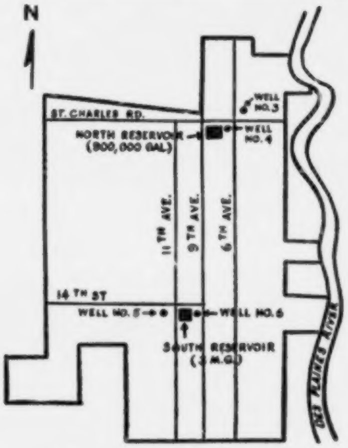

N
150,000 GAL. RESERVOIR
WELL NO. 3
WELL NO. 2
WELL NO. 1
RESERVOIR (1 M.G.)
WELL NO. 4
200,000 GAL RESERVOIR

Figure 6.—*Location of the wells in the city of Elmhurst.*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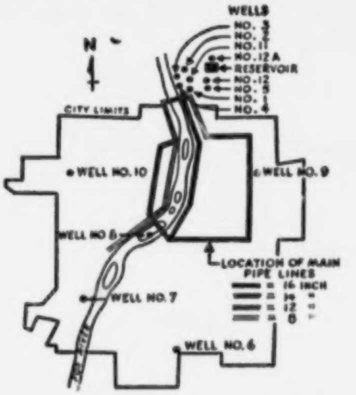
ST. CHARLES RD.
WELL NO. 3
NORTH RESERVOIR (900,000 GAL.)
WELL NO. 4
11TH AVE.
9TH AVE.
6TH AVE.
14TH ST
WELL NO. 5
WELL NO. 6
SOUTH RESERVOIR (3 M.G.)
DES PLAINES RIVER

Figure 7.—*Location of wells in the village of Maywood.*


WELLS
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NO. 2
NO. 11
NO. 12 A
RESERVOIR
NO. 12
NO. 5
NO. 1
NO. 4
N
CITY LIMITS
WELL NO. 10
WELL NO. 9
WELL NO. 8
LOCATION OF MAIN PIPE LINES
= 16 INCH
= 14 "
= 12 "
= 8 "
WELL NO. 7
WELL NO. 6

Figure 8.—*Location of wells in the city of Aurora.*

without any treatment. In 1914 a modern water purification plant was constructed, which was enlarged and improved in 1924.

Oak Park.—Since 1912 the water supply has been obtained from the city of Chicago, the source of this supply being Lake Michigan. This water is chlorinated at Chicago and rechlorinated at Oak Park. Previous to 1912 Oak Park obtained its water supply from deep wells.

Waukegan.—The supply is obtained from Lake Michigan and is treated in a modern water purification plant by aeration, mixing, settling, rapid sand filtration, post aeration, and chlorination. The mixing period is about 5 minutes followed by a period of 3 hours settling; there are 10 rapid sand filters, each having a capacity of 728 gallons per minute. About one grain per gallon of alum is applied for coagulation purposes and 0.3 part per million of chlorine for sterilization. From 1895 to 1929, when the filtration plant was placed in operation, the supply was obtained from Lake Michigan. Previous to 1895 the water supply was obtained from artesian wells.

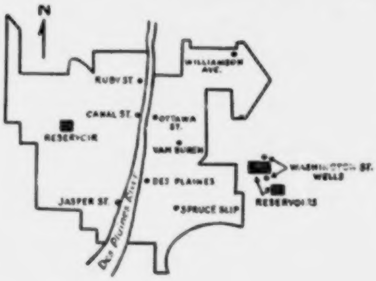

FIGURE 9.—*Location of wells in the city of Joliet.*

TABLE 13.—*Fluoride (F) content of public water supplies in the 8 suburban Chicago communities studied*

Source	Ground water					Lake Michigan		
City or village	Elmhurst	Maywood	Aurora [2]	Joliet	Elgin [3]	Evanston	Oak Park	Waukegan
	Parts per million							
December 1939	1.6	....	1.2	[6] 1.3 [7] 1.2	0.4	0.1	0	0
January 1940	([1])	....	1.2	[6] 1.3 [7] 1.2	.5	0	0	0
February	2.0	0.9	1.1	1.3	.5	0	0	0
March	....	....	1.2	....	.4	0	....	0
April	2.0	[4] 1.1 [5] 1.0	1.2	1.3	.4	0	0	0
May	....	....	1.2	1.2	.5	0	....	0
June	1.6	*1.4 *1.1	1.2	1.4	.5	0	*0 *0	0
July	*1.8 *1.9	*1.0 *1.5	1.2	*1.3 *1.3	.4	.1	*0 *0	0
August	....	....	1.2	....	.5	0	....	0
September	1.9	1.4	1.2	1.3	.4	0	0	....
October	1.6	1.1	1.3	1.2	.5	0	0	*0 *0
November	*1.8 *1.8	*1.1 *1.4	1.3	*1.1 *1.1	.4	0	*0 *0	....
Mean	1.8	[8] 1.2	1.2	1.3	.5	0	0	0

*At times the exigency of other duties prevented the district engineer from collecting the sample during the month specified. When two determinations marked by an asterisk (*) are shown for one month, it indicates that two samples were collected during that month, generally about 2 weeks apart.

[1] Container broken.

[2] All samples collected at main pumping station (wells Nos. 11, 12, 12A, and, recently, 5).

[3] All samples collected at the Elgin City Hall; mixture of water from both ends of town.

[4] North reservoir.

[5] South reservoir.

[6] Ottawa St. well.

[7] Washington St. wells.

[8] There is both presumptive and direct evidence that prior to a few years ago the water supply used in Maywood probably contained 1.4 to 1.6 p. p. m. of fluoride (F). See text.

Other data.—Inasmuch as Oak Park procures its water from the city of Chicago, as does Cicero and numerous other communities in the metropolitan area, three samples were collected during 1939-40 from taps in Cicero having average domestic use, and seven monthly samples were collected during 1940 from a tap in the Chicago distribution system. The three Cicero samples showed 0.1, 0.0, and 0.0 part per million fluoride; all seven of the Chicago samples showed a fluoride content of 0.0 part per million.

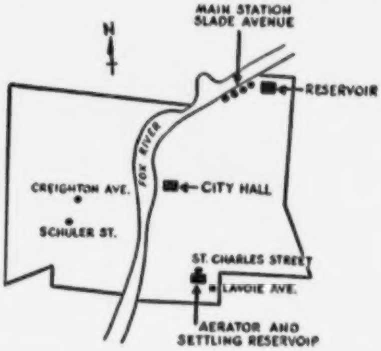

FIGURE 10.—*Location of wells in the city of Elgin.*

The study published in 1937 by Weart and Klassen (*3*) reported that the public water supplies of Elmhurst, Maywood, Aurora, Joliet, and Elgin contained 2.0, 1.6, 1.0, 1.4, and 0.9 parts per million of fluorides, respectively. They noted that in the case of ground water sources a sample from each well then in service was examined, the value reported for each community representing an arithmetical mean of the several determinations.

Chemical analyses of the common water supplies.—Samples of the common water supplies were collected, generally monthly, during 1939-40. The percentage incidence of mottled enamel and

TABLE 14.—*Mineral analyses of the common water supply of each of the 8 suburban Chicago communities studied* [1]

	Elmhurst	Maywood	Aurora	Joliet	Elgin	Evanston	Oak Park	Waukegan
	Parts per million							
Residue on evaporation	737.6	723.2	729.6	566.0	180.0	153.6	152.8	155.2
Loss on ignition	60.8	96.8	107.2	82.8	33.6	34.0	35.6	30.4
Fixed residue	676.8	626.4	622.4	483.2	146.4	119.6	17.2	124.8
Silica (SiO_2)	10.0	11.5	18.4	6.0	26.4	8.0	8.0	4.0
Iron (Fe)	.05	.05	.08	.06	.06	.03	.05	.03
Aluminum (Al)	0	0	0	0	0	.04	0	0
Calcium (Ca)	86.9	20.0	85.5	89.2	14.9	33.7	33.7	34.9
Magnesium (Mg)	25.9	6.1	28.0	30.8	15.9	11.4	11.7	11.5
Sodium and potassium (calculated as Na)	149.8	210.7	130.3	70.5	25.2	3.5	4.4	4.4
Carbonate (CO_3)	0	0	0	0	15.4	0	0	2.4
Bicarbonate (HCO_3)	350.1	351.4	313.5	351.4	103.0	135.4	139.0	139.0
Sulfate (SO_4)	98.1	138.2	48.0	145.8	30.3	23.4	16.8	23.0
Nitrate (NO_3)	.97	.94	1.42	1.59	.33	.62	.46	.89
Chloride (Cl)	180.0	60.0	218.0	31.0	8.0	4.0	5.0	4.0
Phosphate (PO_4)	0	.3	0	0	0	0	0	0
Fluoride (F)	1.8	1.4	1.2	1.2	.4	0	0	0

[1] These samples of water from Aurora and Elgin were received in August 1939; the samples from Elmhurst, Joliet, Evanston, Oak Park, and Waukegan in October 1939, and that from Maywood in June 1940.

Assistant Chemist C. G. Remsburg carried out the determinations other than fluoride, using mostly the methods given in the Standard Methods of Water Analysis of the American Public Health Association. The phosphate was determined colorimetrically by an adaptation of the Benedict and Theis method (J. Biol. Chem., 61: 63 (1924)). The limit of the sensitivity of the procedure used for the fluoride determination may be considered as about 0.1 part per million.

the degree of clinical affection in the age group studied should closely reflect the fluoride (F) content of the water used approximately 9 to 12 years previously. A more precise correlation is possible if single age groupings are studied; even more precision is possible if the signs of mottled enamel in single tooth groupings are considered in relation to the period of enamel calcification as outlined by Logan and Kronfeld. Under these conditions it is possible in the case of certain teeth of the 14-year-old group to estimate the fluoride content of the water used 12 to 14 years previously. Hence, "9 to 12 years previously" is merely an approximation of a prior time period, useful in evaluating certain aspects of the study. On the basis of the presumptive evidence of observed endemic dental fluorosis (mottled enamel), the fluoride content of the public water supplies in use 9 to 12 years ago in these communities was approximately of the same order of fluoride concentration as found in this survey, with the exception of Maywood. At Maywood an incidence of mottled enamel of 33 percent was observed, a degree of affection that would suggest that the water used during the period when these teeth were calcifying had a fluoride (F) concentration of about 1.4-1.6 parts per million, a concentration close to that (1.6 p.p.m.) reported by Weart and Klassen (*3*) in 1937. Physical changes occurring several years ago in certain of the Maywood wells may account for the fact that the mean fluoride (F) content of the samples reported in this paper was 1.2 parts per million.

Klassen states that samples of water collected in 1936 from Maywood wells Nos. 3, 4, 5, and 6 showed a fluoride (F) content of 1.2, 1.3, 1.8, and 1.8 parts per million. These water samples were collected prior to the installation of the softening treatment and the marked changes made in Well No. 6. Raw water samples collected during 1939-40 (not included in table 13) from each of these wells and analyzed by one of us (E.E.) showed fluoride (F) concentrations, in parts per million, as follows: Well No. 3, 1.1 and 1.4; well No. 4, 1.6; well No. 5, 1.3; and well No. 6, 1.1.

A marked difference was noted in the fluoride concentration of the two north wells in contrast to the two south wells, but individual pumping records showing the amount contributed by each of the four village wells for the past 5 years were not available.

The fluoride content of these waters was estimated colorimetrically by means of the zirconium-alizarin reagent (*6*). The results are given in table 13.

As was customary in other quantitative surveys, analyses were made of constituents other than the fluorides. Results of these chemical analyses are given in table 14.

Discussion

General findings.—Marked differences in dental caries experience have been demonstrated in selected population groups (children of continuous residence and continuity of exposure) residing in eight communities in the suburban Chicago area. Considering the relative homogeneity of the population and the sampling method followed, it is difficult from an epidemiological standpoint to attribute these differences to any cause other than the mineral composition of the public water supply. A summary of the findings of this study is shown in table 15.

With respect to the conjectural relationship of the amount of sunlight to dental caries it might be noted that the city (Waukegan) with the highest dental caries experience rate (810) showed the fewest number of cloudy days and was next to Aurora in having the greatest number of clear days.

The characteristic difference in the percentage incidence and dental caries experience rates, proximal caries rates, and amounts of *L. acidophilus* present in the saliva showed no outstanding differences from that observed in the Galesburg-Quincy (Ill.) study (*2*).

Low dental caries experience associated with the use of fluoride waters near the minimal threshold of mottled enamel.—The location of these communities and the fluoride concentrations of their public water supplies make them peculiarly fitted for epidemiological study of the relationship of fluoride concentration in the domestic water supply to the amount of dental caries experience. The most pertinent finding of the study was the disclosure that water supplies, the fluoride concentrations of which were not far from the minimal threshold of endemic dental fluorosis, 1.0 p.p.m. of F (e.g., Aurora, 1.2 p.p.m.), were associated with unusually low dental caries experience rates. Thus, the dental caries inhibitory factor, presumably present in the water and probably fluoride, was operative at levels where mottled enamel per se was of minimal public health and no esthetic significance. On the other hand, the three communities using the fluoride-free waters were all characterized by high dental caries experience. This suggests that fluoride levels even under 1.0 p.p.m. of F influence dental caries experience. The importance of adequate quantitative data respecting dental caries rates in communities whose public

Table 15.—*Summary of dental caries findings in 2,832 selected white children, aged 12–14 years, in 8 suburban Chicago communities in relation to the fluoride (F) content of the public water supply*

City or village	Elmhurst	Maywood	Aurora	Joliet	Elgin	Evanston	Oak Park	Waukegan
Sampling:								
Total number of 12-14-year-old children present at time of sampling	633	873	1,625	1,412	1,030	2,125	1,662	1,354
Number of 12-14-year-old white children whose histories on repeated questioning indicated continuity of exposure and who were examined	170	171	633	447	403	256	329	422
Percentage of the total present who were examined	26.9	19.6	39.0	31.7	39.1	12.0	19.8	31.2
Water supply:								
Source	Deep wells					Lake Michigan		
Hardness in parts per million	323.4	75.0	328.5	349.3	102.6	131.0	132.2	134.4
Mean fluoride (F) content, 1939-40, in parts per million	1.8	[1] 1.2	1.2	1.3	.5	0	0	0
Clinical examination:								
Dental caries experience, permanent teeth, per 100 children examined	252	258	281	323	444	673	722	810
Dental caries experience, proximal surfaces, superior incisors, per 100 surfaces	0.60	0.59	0.78	1.3	4.1	10.7	9.0	17.7
First permanent molar mortality, per 100 children examined	11.8	11.7	14.5	19.5	20.3	42.6	31.0	70.9
Percentage of children with no dental caries experience	25.3	29.8	23.5	18.3	11.4	3.9	4.3	3.1
Percentage incidence of endemic dental fluorosis (mottled enamel)	40.0	33.3	15.0	25.3	4.2	1.6	.6	.2
Bacteriology:								
Percentage of those examined bacteriologically whose salivary *L. acidophilus* counts were:								
Negative and <100	35.7	39.6	38.2	35.6	31.2	15.4	18.2	14.9
30,000 and >	24.7	20.9	25.6	26.6	33.2	41.8	43.8	54.6

[1] There is both presumptive and direct evidence that prior to a few years ago the Maywood water contained probably 1.4-1.6 p. p. m of fluoride (F). See text.

water supplies contain fluoride (F) near or under 1.0 p.p.m. needs no further emphasis.

The fact that low dental caries experience rates were found associated with the use of domestic waters, the fluoride content of which was in the neighborhood of 1.0 p.p.m., naturally brings forth the question of the amount and degree of mildness of the mottled enamel that may follow the continuous use of a domestic water of such concentrations. Examination of an adequate sample of children in communities having the requisites for quantitative evaluation (7) has consistently shown that there is a quantitative relation between the fluoride concentration of the water and the degree of clinical affection (*8*, cf. ogive, fig. 2), the action on the group roughly following general pharmacological observations respecting dosage and effect.

From the data of numerous studies (*9*) one would expect that the examination of an adequate group continuously using a domestic water containing 1.0 p.p.m. of fluoride (F) would show about 88 to 90 percent entirely free of macroscopic signs of mottled enamel. In the remainder (10 to 12 percent), some of the teeth would show the "very mild" types of mottled enamel, generally in the bicuspids and second molars. It is, of course, possible that in some few regions of the United States where climatological conditions such as high mean annual temperature, humidity, wind velocity, etc., may introduce factors conducive to a higher water consumption and higher fluoride intake, a water containing 1.0 p.p.m. of fluoride (F) might be found associated with a higher percentage incidence of affection than that stated.

In communities where the public water supplies contain fluorides just in excess of the minimal threshold (1.0 p.p.m. of F) the reporting of the degree of prevalence as a percentage incidence of the group of children examined actually overstates rather than understates the degree of affection in the group. Aurora may serve as a case in point. Among the 633 children (table 9) an incidence of mottled enamel of 15 percent was recorded, a child being classified as having mottled enamel when a positive diagnosis of mottled enamel was made for as few as two teeth. Now an incidence of 15 percent in the Aurora children should not be construed as meaning that 15 percent of the tooth population of this group showed positive signs of mottled enamel. Actually, of the 16,448 permanent teeth erupted and in position, a positive diagnosis of mottled enamel was possible only in the case of 845 teeth (5.1 percent), 768 (4.7 percent) being "very mild" and 77 (0.4 percent) "mild." It might be noted that no instances of "moderate" (brown stain) or "severe" (discrete or confluent pitting) were observed. A diagnosis of "very mild" is made when a few small white opaque areas are observed involving less than 25 percent of the affected tooth, generally showing on the tip of the cusp of the bicuspids and second molars. The few evidences of dental fluorosis observed were almost exclusively in the bicuspids and second molars; of the 845 teeth diagnosed as positive, only 57, or 6.7 percent, were incisor teeth.

General dietary likeness, exclusive of water.—Regarding the question of the relationship of diet to dental caries, it seems reasonable to assume that the food habitually consumed by these populations follows a general likeness. Hence, considering the sampling method used, it would seem unlikely that the marked differences in dental caries experience were due to differences in the food used in the communities. One would not expect to find gross dietary differences, with the exception of the domestic water, for example, between the children of Oak Park and Maywood, communities within a radius of about a mile. Or again, it would not seem reasonable to assume that the dietary regime (water excluded) of the 633 Aurora children was sufficiently different from that of the 423 Waukegan children to account for a difference in dental caries experience rates of about 188 percent (281 in Aurora and 810 in Waukegan).

Possible relation to the practice of dentistry.—As constituted at present, dentistry's main function might be defined as: (*a*) the clinical control of dental caries by means of fillings; (*b*) the extraction of teeth because of previous attack by dental caries; and (*c*) the attempt to restore, by operative and prosthetic means, teeth lost as a result of dental caries. Thus variations in the intensity of dental caries attack bear important consequential relationships to the practice of denistry, influencing as it does not only the community's dental needs but the kind of dentistry practiced. By referring to figure 3 and studying the filled tooth rate and the missing rate it may be quickly seen how widely different were the amounts of dental service rendered in these different communities. It is important to remember, however, that these comparisons are made among highly selected population groups (children of continuous residence). The influence of such marked dental caries differences in selected children upon the dental caries experience rate of all children in the community has not as yet been determined. For instance, the filled tooth rate in any of the three communities using fluoride-free water is greater than the total dental caries experience in any of the communities (Elmhurst, Maywood, Aurora, Joliet) whose public water supplies contain from 1.2 to 1.8 parts per million of fluoride (F). The influence of the intensity of dental caries attack, manifested by markedly different dental caries experience rates, may be found on further study to have an important bearing on both the proper distribution of dentists and the type of dentistry practiced.

The demonstration of the variation in dental caries experience among selected urban population groups opens up important avenues pertinent to the possibility of partial control of this highly prevalent disease.

SUMMARY

1. A negative correlation between the fluoride (F) concentration of the public water supply and the dental caries experience of children continuously exposed to such waters is reported. A study of eight suburban Chicago communities discloses marked differences in the amount of dental caries. The dental caries experience rates in Elmhurst, Maywood, Aurora, and Joliet, whose public water supplies contain 1.8, 1.2, 1.2, and 1.3, parts per million of fluoride (F), respectively, were 252, 258, 281, and 323, respectively. At Evanston, Oak Park, and Waukegan, using fluoride-free water, the dental caries experience rates were 673, 722, and 810, respectively.

2. Using the proximal surfaces of the four superior permanent incisors as a basis of measurement, there was 14.3 times as much of this type of dental caries in the 1,008 children from Evanston, Oak Park, and Waukegan as in the 1,421 children from Elmhurst, Maywood, Aurora, and Joliet.

3. The differences in the counts of *L. acidophilus* in the saliva corresponded to the differences in the dental caries experience in the groups of communities studied.

4. Considering the relative homogeneity of these urban populations and the sampling method followed, it is difficult from an epidemiological standpoint to ascribe these observed differences to any cause other than the common water supply.

5. The dental caries inhibitory factor, presumably fluoride, was operative at such low concentrations (e.g., 1.2 p.p.m. of F in Aurora) that mottled enamel as an esthetic problem was not encountered.

ACKNOWLEDGMENTS

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Department of Public Health (Division of Dental Health Education, Division of Sanitary Engineering, and Division of Laboratories). Appreciation is also expressed to the District Sanitary Engineers and the City Health Commissioner of Evanston for collecting the monthly water samples shown in table 13.

Acknowledgment is especially made to the numerous educational authorities in each of these eight communities. But for their wholehearted interest and cooperation, this study could not have been made. Special thanks are tendered to Dr. Moreland Emerson, Division of Dental Health Education, whose efforts did much to insure the succes of this study.

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DOMESTIC WATER AND DENTAL CARIES. IV. Effect of Increasing the Fluoride Content of a Common Water Supply on the *Lactobacillus Acidophilus* Counts of the Saliva (Preliminary Report)

By Francis A. Arnold, Jr., H. Trendley Dean, and Elias Elvove

During the past few years epidemiological studies have pointed out an inverse correlation between the amount of dental caries and the presence of fluorides in the domestic water supplies (*1, 2, 3*). Recently Dean, Jay, Arnold, and Elvove (*4*) presented evidence indicating that teeth calcified on a high fluoride water seemingly retained an increased resistance to dental caries even though they had been under the influence of a fluoride-free water for 12 years immediately preceding the examination. The term "fluoride-free" is used in this report for water containing less than 0.2 p.p.m. F—experimental error of F determination is 0.1 p.p.m. These studies and others have indicated the possibility of partially controlling dental caries through the public water supply. This possibility was further enhanced by the findings in the study of 8 suburban Chicago communities (*3*). For instance, at Aurora (Ill.) the dental caries inhibitory factor, presumably present in the water and probably fluoride, was operative at levels (1.2 p.p.m. of F) where mottled enamel per se was of minimal public health and no esthetic significance. At present, however, there is no direct evidence demonstrating whether or not the addition of fluorine to a fluoride-free common water supply will decrease the incidence of dental caries in the community. The epidemiological evidence seemingly justifies the assumption that dental caries activity in humans is markedly inhibited by drinking waters of fluoride concentrations no higher than about the minimal threshold of mottled enamel. Actual field experiments, however, are necessary to determine what age groups might thus benefit, how long ingestion must continue before dental caries activity decreases, and numerous other aspects of this phenomenon as it operates in human populations. An opportunity to study a few of these aspects recently presented itself. This paper is a preliminary report on what may be a long-term study.

Garrettsville, a small village in the northeastern section of Ohio, recently changed its public water supply. Prior to November 1939, the village water supply was obtained principally from three wells, the fluoride (F) content of which was about 0.1 p.p.m. In November 1939, a new well was put into service; the water from this well contains about 1.7 p.p.m. of fluoride (F). Mixture of the water from these wells resulted in a domestic water of about 0.7 p.p.m. F. An opportunity was therefore presented to study children whose domestic water was changed from one practically free of fluorides to a water containing this element in a range previously found (*5*) beneficial to children using such waters throughout life.

DESCRIPTION AND MINERAL COMPOSITION OF THE GARRETTSVILLE WATER SUPPLY

From 1924 to 1939 the water supply for the village was obtained from three drilled wells (Nos. 2, 3, 4), each 8 inches in diamter and approximately 50 feet deep, located about 1.5 miles northeast of the village. The well supply was supplemented during the spring months by water from a spring located in the same general area. This supply was abandoned in 1937. In November 1939 a new well (No. 1), 8 inches in diameter and 210 feet deep, was placed in service. Because of the fluoride content of the water from well No. 1, the water from this source is mixed with water from two of the other wells. On the average day, well No. 1 is pumped 8 hours and delivers approximately 65 gallons per minute and wells Nos. 2 and 3 are pumped 10 hours and deliver 45 to 50 gallons per minute. Since the beginning of the study, well No. 4 has been used only occasionally and at present is not used at all.

Table 1.—*Analyses of the waters used at Garrettsville, Ohio*
[In parts per million [1]]

	Well No. 1	Well No. 2	Well No. 3	Domestic tap
Residue on evaporation	858.4	156.0	165.6	406.4
Loss on ignition	36.8	36.0	36.0	28.0
Fixed residue	821.6	120.0	129.6	378.4
Silica (SiO_2)	18.4	18.0	16.0	20.0
Iron (Fe)	.04	0	.04	.05
Aluminum (Al)	0	0	0	0
Calcium (Ca)	11.4	24.3	26.9	15.7
Magnesium (Mg)	1.4	7.4	8.9	7.4
Sodium and potassium (calculated as Na)	332.2	7.4	7.0	130.2
Carbonate (CO_3)			4.8	
Bicarbonate (HCO_3)	529.5	89.0	68.3	247.6
Sulfate (SO_4)	6.6	23.9	42.8	17.3
Nitrate (NO_3)	0	5.3	.5	2.1
Chloride (Cl)	220.5	2.5	2.0	85.0
Phosphate (PO_4)	0	0	0	0
Fluoride (F)	1.7	[2] .1	[2] .2	[3] .8

[1] The samples from wells Nos. 1 and 2 were received Jan. 15, 1940; those from well No. 3 and the domestic tap were received Feb. 27, 1940. Assistant Chemist C. G. Remsburg carried out the determinations other than fluoride, using mostly the methods given in the Standard Methods of Water Analysis of the American Public Health Association. The fluoride determinations were made by Senior Chemist E. Elvove using the colorimetric Zirconium-Alizarin method (Pub. Health Rep., 48:1219 (1933)).

[2] 3 other monthly samples show 0.1 p. p. m. F.

[3] The mean fluoride (F) content of 12 monthly samples of the domestic tap was 0.7 p. p. m. (range 0.5-0.8).

The water obtained from these wells is treated in an iron-removal plant. This plant comprises an aerator, a settling tank divided into four units operating in series, and two gravity type rapid sand filters. Water is pumped directly from the wells to the treatment plant from where it flows by gravity to the clear well. High service pumping equipment pumps water directly from the clear well to the storage reservoir. All the water supplied to the town comes from this one storage reservoir. The present water consumption of the village ranges from 70,000 to 105,000 gallons daily.

PLAN OF STUDY

The present study group is composed of white public school children living within the corporate limits of the village and using the public water supply for all domestic purposes. Only children in the 4th to 10th grades were included in the study. Their ages ranged from 9 to 17 years at the beginning of the study, the average being 13 years. The original group included a total of 132 children, but due to absentees, migrations, and other uncontrollable factors, only 109 were followed for the complete year of study. All 109 children were living in Garrettsville prior to the change of water.

Bacteriological Study and Results

The technique followed in obtaining *L. acidophilus* counts was similar to that used in other studies in this series (*2, 3, 4*). Paraffin stimulated saliva samples were collected between 10:30 and 11:30 a.m. The time allowed for collection of saliva was approximately 5 minutes in all cases. The dilution used throughout was 1 cc. saliva to 4 cc. broth; 0.1 cc. of the broth and saliva mixture was placed on tomato juice agar, pH 5.0. All samples were collected at the school and were taken that same day to the Institute of Pathology, Western Reserve University, Cleveland, Ohio, where they were plated and incubated. During the year 1940–41, eight separate saliva samples were collected from each child (two in May, three in November, three in April) and counts determined. The results of these eight examinations are shown in table 2.

Previous studies in this series (*2, 3, 4*) have indicated a correlation between the dental caries experience of the group and the *L. acidophilus* counts. In these studies group differences were found principally in the percentages of low counts (negative and less than 100) and high counts (30,000 and over). The results of one of these studies (*3*) present evidence that there is a group difference in *L. acidophilus* counts of saliva from children who were born and reared in a community where the water contained 0.5 p.p.m. fluorides (F) (Elgin, Ill.) when compared with counts from children who were continuously exposed to a fluoride-free water (Waukegan, Ill.). In order to compare the bacteriological results of the present study obtained at three different periods of the year with the results obtained at Elgin and Waukegan, Ill., the percentage of low and high *L. acidophilus* counts are shown graphically in figure 1. In order to make the data comparable the percentages shown for the low and high *L. acidophilus* counts at Garrettsville are those obtained on the first day of each study period.

Clinical Examination and Results

In order to have some measure of the prevalence of dental caries in this group of children, a complete dental examination was made in December 1940. This examination was made by use of mouth mirror and explorer in a well-lighted schoolroom with the child seated and facing a window. The criteria for making a diagnosis of dental caries were the same as used in previous studies (*2, 3*).

Table 2.—*Summary of the distribution of oral L. acidophilus in 8 individual saliva samples from a group of 109 school children, Garrettsville, Ohio, during a 1-year period*

Date of collection	Distribution of children according to the estimated number of oral *L. acidophilus* per cubic centimeter of saliva								
	Negative	Less than 100	100 to 1,000	1,000 to 3,000	3,000 to 12,000	12,000 to 21,000	21,000 to 30,000	30,000 and over	Total
NUMBER									
May 14, 1940	6	11	3	3	8	12	8	58	109
May 15, 1940	6	12	6	3	6	11	8	57	109
Nov. 12, 1940	11	4	10	2	9	16	4	52	108
Nov. 13, 1940	11	3	10	3	9	5	6	61	108
Nov. 14, 1940	13	4	5	4	6	5	5	63	105
April 14, 1941	8	7	2	0	8	7	5	68	105
April 15, 1941	9	3	1	1	13	4	3	72	106
April 16, 1941	11	1	1	2	10	6	4	71	106
PERCENT									
May 14, 1940	5.5	10.1	2.8	2.8	7.3	11.0	7.3	53.2	....
May 15, 1940	5.5	11.0	5.5	2.8	5.5	10.1	7.3	52.3	....
Nov. 12, 1940	10.2	3.7	9.3	1.9	8.3	14.8	3.7	48.1	....
Nov. 13, 1940	10.2	2.8	9.3	2.8	8.3	4.6	5.6	56.5	....
Nov. 14, 1940	12.4	3.8	4.8	3.8	5.7	4.8	4.8	60.0	....
April 14, 1941	7.6	6.7	1.9	.0	7.6	6.7	4.8	64.8	....
April 15, 1941	8.5	2.8	.9	.9	12.3	3.8	2.8	67.9	....
April 16, 1941	10.4	.9	.9	1.9	9.4	5.7	3.8	67.0	....

In computing the dental caries experience no tooth was counted more than once, even though it had a filling on one surface and an untreated lesion on another. Any tooth showing evidence of a filling was classified as a filled tooth. The principal findings of the clinical examination are shown in table 3.

TABLE 3.—*Summary of the clinical findings observed in an examination of 107 white school children, aged 9 to 17 years, at Garrettsville, Ohio*

Dental caries experience, permanent teeth						Macroscopic evidence of endemic dental fluorosis		
Teeth filled (past dental caries) (a)	Teeth with untreated dental caries (b)	Teeth, extraction indicated (c)	Teeth, missing (d)	Total (a+b+c+d)	Proximal surfaces 4 superior incisors	Absent		Present
						Normal	Questionable	Very mild
Number per 100 children examined					Number per 100 surfaces	Percent		Percent
361	284	16.8	46.7	708	12.7	83.2	15.0	1.8

DISCUSSION

The results of the single clinical examination given in table 3 record the dental caries experience of the permanent teeth of the study group up to the time of examination. Considering the average age of these children when examined (13.6 years) and their caries experience rate (708), it seems logical to assume that they have had a somewhat similar caries experience to that observed in children born and reared in communities in which the water is practically free of fluorides (*3*). The dental caries experience rate of 1,008 children examined in Evanston, Oak Park, and Waukegan, Ill., in the study cited was 746. However, it should be noted that the range of ages in the present study (9 to 17 years) is different from that of the children referred to in these three cities. The low incidence of dental fluorosis (1.8 percent) is similar to the sporadic instances of "very mild" mottled enamel found in children who have calcified their teeth on water which is practically free of fluorine. In view of this evidence one might assume that up to the time of change in water supply this group of children at Garrettsville had an oral environment comparable to children born and reared in communities with practically fluoride-free waters.

In order to obtain direct evidence of a change of caries activity in a study of this nature, especially with such limited numbers, it will be necessary to make repeated clinical examinations. Such a plan necessarily requires sufficient time to elapse between examinations so that comparisons can be made on the basis of similar age groups with like exposure to risk of caries under the changed environment (new water supply). A presumptive indication of any significant change in caries activity of the study group might be gained by use of *L. acidophilus* counts as a measure of caries activity. Previous studies in this series indicate that a negative correlation exists between the caries activity of a group population, as measured by the percentage distribution of *L. acidophilus* counts, and the presence of fluorides in the public water supplies. Attention may be called, however, to the fact that in the studies cited only children 12 to 14 years of age, *who had been continuously exposed to the respective waters throughout life,* were included in the study groups.

In view of the clinical results (as to the amount of dental caries) in the present study group, and the bacteriological results in previous studies, one might expect that had there been an immediate beneficial change in caries activity between the date of change of water supply (November 1939) up to the beginning of the study (May 1940) the *L. acidophilus* counts would be changed. In other words, there would have been an increase in the number of low counts (0 or less than 100) and a decrease in the high counts (30,000 and over). However, the counts obtained in May 1940 indicate that no such change had occurred. The *L. acidophilus* counts at the beginning of the study would indicate that the caries activity of the group was similar to that found at Waukegan, Ill., (*3*) where the children studied had been continuously exposed to a fluoride-free water and had a similar high dental caries experience rate.

If a decrease in caries activity of the

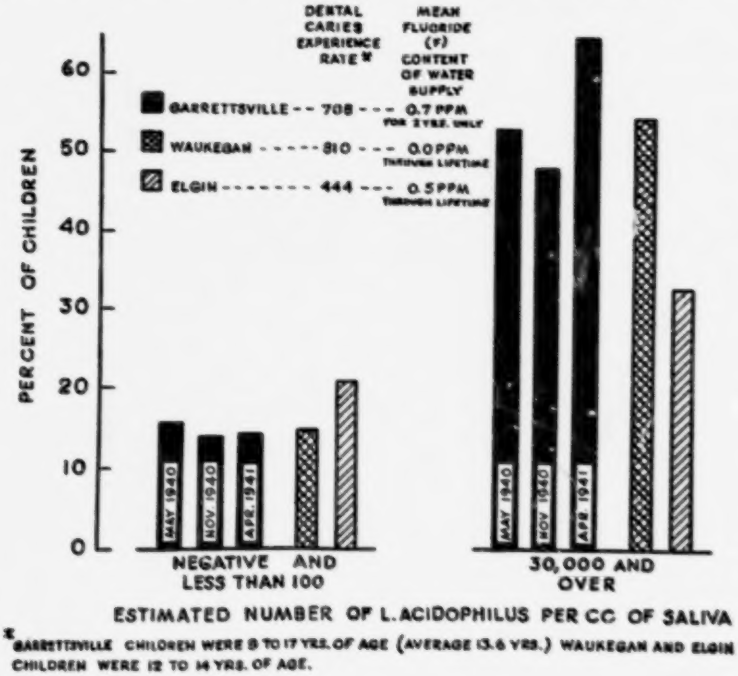

FIGURE 1.—*A comparison of the low and high* L. acidophilus *counts obtained at Garrettsville, Ohio, with counts obtained at Waukegan and Elgin, Ill. The results shown for Garrettsville are those obtained on the first day of each visit during the study year.*

study group occurred during the year, the bacteriological results might be expected to have given some indication of this change. Had there been an increase in the number of low counts or a significant decrease in the number of high counts, a beneficial change in caries activity might have been assumed. This bacteriological change did not occur. If any change in caries activity might be inferred from the minor changes in the bacteriological counts, it would suggest a change toward a higher percentage of the children having active dental caries. According to the results shown in table 2 there was an average of 16.1 percent low counts in May 1940, and 12.3 percent low counts in April 1941; however, at present these changes cannot be considered significant.

The results obtained so far in this study might be interpreted as showing that increasing the fluoride content of a public water supply from 0.1 to 0.7 p.p.m. F will have little influence on dental caries activity for at least the first 2 years. In comparing the results of this study with previous studies in this series, one might infer that it is necessary that the individual be born and reared in a community having water of this approximate fluoride content in order to obtain any beneficial effects. It should be noted, however, that the group of children in Garrettsville have been using this new water supply for a comparatively short time and it is possible that the *L. acidophilus* counts obtained represent caries activity in lesions which started prior to the change in water.

SUMMARY

There are presented preliminary results obtained in a study of 109 public school children who have been exposed for about 2 years to a domestic water which has been increased in fluoride (F) content from about 0.1 to 0.7 p.p.m.

A single clinical examination indicated that the dental caries experience rate of these children was similar to the rates reported in previous studies on children who were born and reared in communities where the domestic water is practically free of fluorides.

There is no indication according to the percentage distribution that the increased fluoride content of the water supply has as yet influenced the *L. acidophilus* counts. Such results might indicate that at least for the first 2 years increasing the fluoride (F) content of a public water supply from 0.1 p.p.m. to approximately 0.7 p.p.m. would result in little, if any, decrease in caries activity of children whose teeth had calcified and had been exposed for a number of years to a water practically free of fluorides. However, the relatively unchanged *L. acidophilus* counts in this group may be the result of dental caries activity in lesions which had started prior to the change of water and were still active, and subsequent clinical examinations may possibly show that fewer new carious lesions have developed since the introduction of the new water supply.

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The authors desire to express their appreciation to J. A. Ricker, superintendent of water works, Garrettsville, Ohio, for his cooperation in maintaining the mixture of well waters at a constant level during this study; also to J. O. Fox, superintendent of schools, for continued cooperation throughout the study. Thanks are also expressed to officials of the Ohio Department of Health and the Portage County Health Department. Special thanks are likewise expressed to the Institute of Pathology, Western Reserve University, for providing the facilities for conducting the bacteriological studies.

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DOMESTIC WATER AND DENTAL CARIES. V. Additional Studies of the Relation of Fluoride Domestic Waters to Dental Caries Experience in 4,425 White Children, Aged 12 to 14 Years, of 13 Cities in 4 States

By H. Trendley Dean, Francis A. Arnold, Jr., and Elias Elvove (with clinical examinations by David C. Johnston and Edwin M. Short)

Recent reports (*1*, *2*) have pointed out an inverse relationship between the fluoride content of the public water supply and the dental caries experience of those children continuously using such waters throughout life. A further study of this phenomenon has been made in 21 cities of 4 States where the public water supplies varied not only in fluoride content but with respect to other mineral constituents as well.

A portion of this investigation—a study of 2,832 children in 8 suburban Chicago communities—has recently been reported (*3*). The present paper records the findings observed in 4,425 additional children of 13 other cities, bringing the total number of white urban school children, aged 12 to 14 years, examined to 7,257. All were examined by one or the other of two dental examiners (D. C. J. and E. M. S.), each examining approximately an equal number of children in each city.

TABLE 1.—*Statistics with respect to the composition of the population of the 13 cities studied (census of 1930)*

City	Population								
	Total	White	Negro	Other races[1]	Total	White	Negro	Other races[1]	Percent native white of white population
	Number				Percent				
Galesburg, Ill	28, 830	27, 671	891	268	100. 0	95. 98	3. 09	0. 93	92. 0
Colorado Springs, Colo	33, 237	31, 828	965	444	100. 0	95. 76	2. 90	1. 34	93. 2
East Moline, Ill	10, 107	9, 462	470	175	100. 0	93. 62	4. 65	1. 73	78. 9
Kewanee, Ill	17, 093	16, 720	278	95	100. 0	97. 82	1. 63	. 55	85. 9
Pueblo, Colo	50, 096	45, 131	1, 305	3, 660	100. 0	90. 10	2. 60	7. 30	89. 5
Marion, Ohio	31, 084	30, 690	387	7	100. 0	98. 73	1. 25	. 02	97. 7
Lima, Ohio	42, 287	40, 848	1, 422	17	100. 0	96. 60	3. 36	. 04	96. 4
Quincy, Ill	39, 241	38, 062	1, 145	34	100. 0	97. 00	2. 92	. 08	96. 2
Middletown, Ohio	29, 992	27, 186	2, 805	1	100. 0	90. 64	9. 35	. 01	95. 7
Zanesville, Ohio	36, 440	34, 659	1, 776	5	100. 0	95. 11	4. 87	. 02	97. 2
Portsmouth, Ohio	42, 560	40, 658	1, 891	11	100. 0	95. 53	4. 44	. 03	98. 4
Elkhart, Ind	32, 949	32, 394	539	16	100. 0	98. 31	1. 64	. 05	94. 9
Michigan City, Ind	26, 735	25, 533	1, 071	131	100. 0	95. 50	4. 00	. 50	87. 1

[1] Although the Negro was excluded from this study because of the possibility of a racial difference in attack by dental caries, no attempt was made to eliminate children of "Other races." This segment of the population comprised a relatively small percentage of the general population (1.2 percent) of the 13 cities studied, and it seemed unnecessary to eliminate the occasional child who may have belonged in this classification. They are, accordingly, included with the white children in the tables that follow in this paper. Persons of Mexican birth or parentage who were not definitely reported as white or Indian were designated "Mexican" in the 1930 census and included in the general class of "Other races." In previous censuses most of the Mexicans have been classified as white. Of the 4,594 persons listed in this column, East Moline and Kewanee (Ill.) excluded, 4,356, or close to 95 percent, were Mexicans.

The same methods used in the study of the 8 suburban Chicago communities with respect to age, sex, color, continuity of exposure, and other epidemiological factors discussed in detail in that report (*3*) were followed in the study of the 13 additional cities which form the basis of this paper. For purposes of summarizing these field findings in the discussion which follows later in this paper, certain data from the study of 8 suburban Chicago communities will be included with that of the 13 additional cities which form the basis of this report.

The study of the eight suburban Chicago communities showed, in part, that the continuous use of a domestic water, the fluoride content of which was close to the minimal threshold of endemic dental fluorosis (mottled enamel), was associated with a relatively low dental caries experience. For example, at Aurora (Ill.), where the domestic water contained 1.2 p.p.m. of fluoride (F) and where a relatively low dental caries prevalence was recorded, mottled enamel as an esthetic problem was not encountered. Strong presumptive evidence suggests that the factor or factors responsible for this increased freedom from dental caries is the fluoride content of the domestic water; the fact that it was operative at concentration levels so low that mottled enamel ceased being an accessory complication was a finding of first importance.

Where it was also apparent that all three cities using fluoride-free waters were characterized by relatively high dental caries prevalence, it seemed likely that fluoride levels under 1.0 p.p.m. of fluoride (F) might also influence the intensity of dental caries attack. The term "intensity of dental caries attack" as used in this paper may be defined as the force of the factors responsible for the initiation (or inhibition) and rate of progress (or quiescence) of the dental caries process. This force of attack (or force of resistance) is subject to considerable change dependent upon varying circumstances. The study was therefore extended to include certain additional cities whose water supplies contained fluorides in these lower concentration levels.

This paper describes studies made in Illinois, Indiana, Ohio, and Colorado, and reports the amount of dental caries experience found associated with the continuous use of common water supplies obtained from Lake Michigan, the Mississippi, Ohio, and Arkansas Rivers, from deep wells of different fluoride concentration and mineral composition, and in one instance from melted snow high on Pike's Peak (Colorado Springs).

This survey immediately followed the study of the eight suburban Chicago communities previously reported (*3*), all clinical examinations being made by the same two dental examiners. With the exception of Kewanee (Ill.), all examinations were made during 1940. Examinations in Kewanee were made in December 1939. The chronological order in which the clinical examinations were made in 1940 was as follows: Zanesville and Portsmouth (January); Middletown (January-February); Marion and Lima (February); Elkhart and Michigan City (March); Colorado Springs (April); Pueblo (May); Quincy (September); Galesburg (September-October); and East Moline (October). The order in which these cities were studied was: Kewanee (Ill.); Zanesville, Portsmouth, Middletown, Marion, and Lima (Ohio); Elkhart and Michigan City (Ind.); Colorado Springs and Pueblo (Colo.), and Quincy, Galesburg, and East Moline (Ill.). The children of Galesburg and Quincy were examined in December 1939, by Dental Surgeon H. T. Dean, U.S. Public Health Service,

TABLE 2.—*A 15-year summary (1925–39) of the actual, or estimated, average number of "clear days" per year recorded for the 13 cities studied*

(From Climatological Data, Weather Bureau)

City	Number of days		Number of clear days[2] (A+B)	Number of years of observation	Clear days		Increasing order of—	
	Clear[1]	½ partly cloudy[1]			Average per year $\left(\frac{C}{D}\right)$	Percent $\frac{E}{365}$	Dental caries experience	Percent of clear days[2]
	A	B	C	D	E			
Galesburg, Ill	2, 625	556	3, 181	15	212. 1	58. 1	1	9
Colorado Springs, Colo	3, 040	791	3, 831	15	255. 4	70. 0	2	13
East Moline, Ill	2, 053	810	2, 863	15	190. 9	52. 3	3	3
Kewanee, Ill	2, 612	625	3, 237	15	215. 8	59. 1	4	10
Pueblo, Colo	2, 489	1, 094	3, 583	15	238. 9	65. 5	5	12
Marion, Ohio	1, 415	1, 004	2, 419	15	161. 3	44. 2	6	1
Lima, Ohio	2, 190	707	2, 897	15	193. 1	52. 9	7	4
Quincy, Ill	2, 508	563	3, 071	15	204. 7	56. 1	8	6
Middletown, Ohio	2, 688	695	3, 383	15	225. 5	61. 8	9	11
Zanesville, Ohio	2, 491	603	3, 094	15	206. 3	56. 5	10	7
Portsmouth, Ohio	2, 818	360	3, 178	15	211. 9	58. 1	11	8
Elkhart, Ind	1, 811	758	2, 569	15	171. 3	46. 9	12	2
Michigan City, Ind	2, 615	454	3, 069	15	204. 6	56. 1	13	5
Total	31, 355	9, 020	40, 375	195	207. 1	56. 7	----------	----------

[1] "Clear days" and "partly cloudy days" as defined by the Weather Bureau. (See footnote 6, p. 1155.)
[2] "Clear days" as defined in text.

and Dr. O. S. Hoag, Illinois Department of Public Health, as reported in reference (*2*). Because of the variation in diagnostic criteria of different dental examiners, the children of Galesburg and Quincy were again examined in September and October 1940, by Assistant Dental Surgeons (R) Johnston and Short in order that all dental caries experience reported in this paper might be on a comparable basis of diagnostic standards.

In the tables to follow these 13 cities will be listed in accordance with the increasing order of observed dental caries experience rates which are: Galesburg (Ill.); Colorado Springs (Colo.); East Moline (Ill.); Kewanee (Ill.); Pueblo (Colo.); Marion, Lima, and Middletown (Ohio); Quincy (Ill.); Zanesville and Portsmouth (Ohio), and Elkhart and Michigan City (Ind.).

Population of cities studied.—Population statistics with respect to the 13 cities studied are given in table 1. As the study was limited to white school children, the percentage of native white was computed on the basis of the total white population, not the total population. Briefly, it shows that at Galesburg, Colorado Springs, East Moline, Kewanee, Pueblo, Marion, Lima, Middletown, Quincy, Zanesville, Portsmouth, Elkhart, and Michigan City the percentage of the native white of the white population was: 92.0, 93.2, 78.9, 85.9, 89.5, 97.7, 96.4, 96.2, 95.7, 97.2, 98.4, 94.9, and 87.1, respectively.

TABLE 4.—*Prevalence of dental caries experience, permanent teeth, in 4,425 selected white school children, aged 12 to 14 years, classified by cities, according to: (a) age distribution, the number and percent of children showing dental caries experience, and (b) the amount of dental caries experience*

City	Number of children examined				Children showing—		Permanent teeth showing dental caries experience
	All ages	Age in years, last birthday			Dental caries experience	No dental caries experience	
		12	13	14			
	Number						
Galesburg, Ill	273	89	100	84	197	76	643
Colorado Springs, Colo	404	143	137	124	289	115	994
East Moline, Ill	152	54	58	40	121	31	461
Kewanee, Ill	123	42	40	41	101	22	422
Pueblo, Colo	614	188	253	173	549	65	2,528
Marion, Ohio	263	88	78	97	248	15	1,461
Lima, Ohio	454	147	148	159	444	10	2,962
Middletown, Ohio	370	116	141	113	363	7	2,601
Quincy, Ill	330	100	125	105	322	8	2,329
Zanesville, Ohio	459	147	175	137	447	12	3,366
Portsmouth, Ohio	469	128	177	164	463	6	3,622
Elkhart, Ind	278	79	122	77	274	4	2,289
Michigan City, Ind	236	77	84	75	236	0	2,448
	Percent						Number per 100 children examined
Galesburg, Ill		32.6	36.6	30.8	72.2	27.8	236
Colorado Springs, Colo		35.4	33.9	30.7	71.5	28.5	246
East Moline, Ill		35.5	38.2	26.3	79.6	20.4	303
Kewanee, Ill		34.2	32.5	33.3	82.1	17.9	343
Pueblo, Colo		30.6	41.2	28.2	89.4	10.6	412
Marion, Ohio		33.5	29.6	36.9	94.3	5.7	556
Lima, Ohio		32.4	32.6	35.0	97.8	2.2	652
Middletown, Ohio		31.4	38.1	30.5	98.1	1.9	703
Quincy, Ill		30.3	37.9	31.8	97.6	2.4	706
Zanesville, Ohio		32.0	38.1	29.9	97.4	2.6	733
Portsmouth, Ohio		27.3	37.7	35.0	98.7	1.3	772
Elkhart, Ind		28.4	43.9	27.7	98.6	1.4	823
Michigan City, Ind		32.6	35.6	31.8	100.0	0.0	1,037

Climatological data (sunshine).—Weather Bureau reports list the number of clear, partly cloudy, and cloudy days as recorded at stations located at or near the cities included in this study. A day is classified clear, partly cloudy, or cloudy on the basis of hourly estimations, sunrise to sunset, as follows: Clear, sky averages three-tenths or less obscured; partly cloudy, sky averages four-tenths to seven-tenths inclusive, obscured; and cloudy, sky averages more than seven-tenths obscured. To estimate roughly the amount of sunshine present in these cities the Weather Bureau recordings for clear, partly cloudy, and cloudy days were divided dichotomously into "clear" and "nonclear" days, the term "clear days" as used in this paper being defined as the number of clear days reported by the Weather Bureau plus one-half the number of days listed as partly cloudy. The average number of "clear days" and the percentage of "clear days" per year was determined for the 15-year period covering approximately the life span of the children examined and is shown in table 2. Certain of these values were obtained from stations located in the city studied. Where this was not possible the values recorded at the nearest seemingly comparable city are given and in some cases where two stations were about equidistant, an arithmetic mean of the reports from both stations. These data are shown in table 2.

In connection with the alleged in-

TABLE 3.—*Summary of data with relation to continuity of exposure to the public water supply of 4,425 selected white children, aged 12 to 14 years, residing in 13 cities of Illinois, Indiana, Ohio, and Colorado*

City	Number of 12- to 14-year-old children in attendance on the day study group was selected	Number of 12- to 14-year-old white children whose histories on repeated questioning [1] indicated continuity of exposure and who were examined	Percentage of the total present who were examined
Galesburg, Ill	918	273	29.7
Colorado Springs, Colo	1,444	404	28.0
East Moline, Ill	352	152	43.2
Kewanee, Ill	522	123	23.6
Pueblo, Colo	1,412	614	43.5
Marion, Ohio	1,010	263	26.0
Lima, Ohio	1,411	454	32.2
Middletown, Ohio	1,013	370	36.5
Quincy, Ill	1,063	330	31.0
Zanesville, Ohio	1,248	459	36.8
Portsmouth, Ohio	1,228	469	38.2
Elkhart, Ind	942	278	29.5
Michigan City, Ind	654	236	36.1
Total	13,217	4,425	33.5

[1] About 14 percent of the group (5,127) for whom sampling cards were made out were not examined. The detailed subsequent questioning which disclosed breaks in continuity of exposure warranting elimination from the study accounted for about half of the cases excluded (7 percent) and these together with those absent on the day of examination (2.5 percent), colored (4 percent), and miscellaneous comprised the 14 percent referred to.

fluence of sunshine on dental caries prevalence, it might be noted that cities characterized by high dental caries experience, e.g., Portsmouth and Middletown, show percentages of "clear days" as high or higher than that of Galesburg, a city where a very low dental caries prevalence was observed.

Selection of study groups.—The study groups were selected in a manner described in detail in a previous report (*3*). *The groups examined generally represent all 12-, 13-, and 14-year-old white public school children continuously exposed to the variable under investigation (the public water supply).* All public schools in the community having a seventh, eighth, or ninth grade were included in the study, but no effort was made to locate 12- to 14-year-old children in grades other than the three specified, with the exception of those instances where an appreciable number of children of the age group studied were in the sixth grade.

At Kewanee, Zanesville, and Portsmouth the selection was done by the same individual (H. T. D.) as in the study of the eight suburban Chicago communities. In the other 10 cities the selection was carried out by the two dental examiners (D. C. J. and E. M. S.) in a manner similar to that followed in the cities previously studied. Table 3 shows the number of 12- to 14-year-old pupils present the day the study group was selected and the number and percentage of these whose histories on repeated questioning indicated continuity of exposure and who were examined.

Clinical examinations.—All clinical examinations were made in a manner similar to that followed in the study of the eight suburban Chicago communities (*3*), and by the same two dental examiners.

CLINICAL FINDINGS

In table 4 are shown the number of children examined, the age distribution, the number and percentage of children with one or more permanent teeth showing dental caries experience, the number and percentage of children with no dental caries experience, and the total dental caries experience (permanent teeth) observed in each city. All data in the tables refer to permanent teeth only.

The amount of dental caries in the populations studied is expressed quantitatively in terms of the total caries experience of the group. This method of reconstituting the complete caries experience in the permanent teeth of children with a fair degree of precision has been described by Klein and Palmer in Pub. Health Bulletin No. 239, reference (*4*), this paper, and other publications. Although the method of estimating the total amount of past and present dental caries described in this paragraph deals with teeth *per se*, similar criteria may be applied in measuring dental caries prevalence for tooth surfaces as may be seen in table 7 of this paper. This is determined by totaling the number of filled teeth (past dental caries), the number of teeth with untreated dental caries (irrespective of the number of defects per tooth), the number of teeth indicated for extraction, and the number of missing teeth. In this study third molars are excluded from consideration; the occasional instance of teeth lost by accident or extracted because of malposition is also excluded. In computing this index, no single tooth was counted more than once even though one surface may have shown a carious lesion and another surface a filling. A tooth showing both an untreated lesion and a filling was counted as a "filled tooth." To express the dental caries experience (teeth) in terms of a rate per hundred children examined, the sum of the four aggregates referred to is divided by the number of children examined and the quotient multiplied by 100. These data are given in table 4.

In addition to reporting the total dental caries experience, it seems desirable to show how much each of the following items contributed to the rates shown: Filled teeth (past dental caries), teeth with untreated dental caries, teeth in which extraction is indicated, and missing teeth (teeth lost because of accident or extracted because of malposition excluded). These data are shown in table 5.

Proximal dental caries.—Outstanding differences in the amount of dental caries in the proximal surfaces of the four superior permanent incisors have been reported (*2, 3*). For instance, in the study of eight suburban Chicago communities, there was 14.3 times as much of this type of caries in the 1,008 children using fluoride-free waters (Evanston, Oak Park, and Waukegan) as was observed in the 1,421 children using a water whose fluoride (F) content exceeded 1.0 p.p.m. (Elmhurst, Maywood, Aurora, and Joliet).

The dental caries experience of the eight proximal surfaces of the four superior permanent incisors in the children of the 13 cities included in this report are shown in table 6.

TABLE 5.—*Summary of the dental caries experience in the permanent teeth of 4,425 white school children, aged 12 to 14 years, of 13 cities in Illinois, Indiana, Ohio, and Colorado, classified on the basis of filled teeth (past dental caries), teeth with untreated dental caries, extraction indicated, and missing teeth (presumably because of dental caries)*

City	Children examined	Dental caries experience, permanent teeth				
		Filled teeth (past dental caries) (a)	Teeth with untreated dental caries (b)	Extraction indicated (c)	Missing teeth (d)	Total (a+b+c+d)
(A) NUMBER						
Galesburg, Ill	273	217	385	15	26	643
Colorado Springs, Colo	404	240	734	5	15	994
East Moline, Ill	152	103	334	9	15	461
Kewanee, Ill	123	77	308	9	28	422
Pueblo, Colo	614	377	2,017	48	86	2,528
Marion, Ohio	263	213	1,175	17	56	1,461
Lima, Ohio	454	653	2,037	59	213	2,962
Middletown, Ohio	370	653	1,687	41	220	2,601
Quincy, Ill	330	917	1,167	75	170	2,329
Zanesville, Ohio	459	908	1,961	156	341	3,366
Portsmouth, Ohio	469	1,202	2,041	108	271	3,622
Elkhart, Ind	278	789	1,404	25	71	2,289
Michigan City, Ind.	236	770	1,463	61	154	2,448
(B) NUMBER PER 100 CHILDREN EXAMINED						
Galesburg, Ill		79.5	141.0	5.5	9.5	236
Colorado Springs, Colo		59.4	181.7	1.2	3.7	246
East Moline, Ill		67.8	219.7	5.9	9.9	303
Kewanee, Ill		62.6	250.4	7.3	22.8	343
Pueblo, Colo		61.4	328.5	7.8	14.0	412
Marion, Ohio		81.0	446.8	6.5	21.3	556
Lima, Ohio		143.8	448.7	13.0	46.9	652
Middletown, Ohio		176.5	455.9	11.1	59.5	703
Quincy, Ill		277.9	353.6	22.7	51.5	706
Zanesville, Ohio		197.8	427.2	34.0	74.3	733
Portsmouth, Ohio		256.3	435.2	23.0	57.8	772
Elkhart, Ind		283.8	505.0	9.0	25.5	823
Michigan City, Ind		326.3	619.9	25.8	65.3	1,037

Marked differences were noted in the amount of this type of dental caries be-

TABLE 6.—*Summary of the findings relative to the dental caries experience, proximal surfaces, of the four superior permanent incisors of 4,425 selected white school children, aged 12 to 14 years, of 13 cities*

City	Number of children examined	Dental caries experience, proximal surfaces, superior permanent incisors				
		Children showing 1 or more surfaces with dental caries experience		Total number of proximal surfaces [1]	Number of proximal surfaces with dental caries experience [2]	Dental caries experience per 100 surfaces
		Number	Percent			
(A) CITIES WHOSE WATER SUPPLIES CONTAINED 0.5 P. P. M. OR MORE OF F.						
Galesburg, Ill	273	9	3.3	2,162	10	0.46
Colorado Springs, Colo	404	5	1.2	3,186	10	.31
East Moline, Ill	152	2	1.3	1,214	2	.16
Kewanee, Ill	123	6	4.9	976	14	1.4
Pueblo, Colo	614	12	2.0	4,854	23	.47
Total	1,566	34	2.2	12,392	59	.48
(B) CITIES WHOSE WATER SUPPLIES CONTAINED LESS THAN 0.5 P. P. M. OF F.						
Marion, Ohio	263	34	12.9	2,076	69	3.3
Lima, Ohio	454	52	11.5	3,580	113	3.2
Middletown, Ohio	370	78	21.1	2,904	205	7.1
Quincy, Ill	330	100	30.3	2,596	291	11.2
Zanesville, Ohio	459	136	29.6	3,618	412	11.4
Portsmouth, Ohio	469	136	29.0	3,704	386	10.4
Elkhart, Ind	278	86	30.9	2,208	248	11.2
Michigan City, Ind	236	101	42.8	1,874	339	18.1
Total	2,850	723	25.3	22,560	2,063	9.1
Grand total	4,425	757	17.1	34,952	2,122	6.1

[1] Teeth lost by accident, unerupted, extracted because of malposition, and proximal surfaces restored by prosthesis (inlays, ¾ crowns, etc.) because of traumatic injury, excluded. The maximum possible number of surfaces in a population of this size (4,425) is 35,400. The number of surfaces excluded for the reasons stated was 448, or approximately 1.3 percent.

[2] Teeth listed as "extraction indicated" and "missing," not covered by the foregoing exceptions, were assumed to have had both surfaces attacked by caries and were so counted. These, together with the number of filled proximal surfaces (past caries) and the number of proximal surfaces with untreated carious lesions constitute the complete caries experience.

tween the eight cities whose public water supplies contained less than 0.5 part per million of fluoride (F) and the five cities whose water supplies contained 0.5 part per million or more. When comparisons are made on the basis of affected tooth surfaces, the rate in the cities with the lower fluoride water supplies was about 19 times as high as in the cities with the higher fluoride content; on the child-unit basis of comparison there was 11.5 times as much in the former cities as in the latter.

First permanent molar mortality.—The first permanent molar mortality rate for each of these 13 cities was computed. Knutson and Klein (Pub. Health Rep., *53:* 1021–1032 (June 24, 1938) define tooth mortality as referring to "not only extracted permanent teeth but also those which are indicated for extraction and still present in the mouth." First permanent molar mortality rates reported in table 7 were computed in accordance with this definition. As tooth mortality may to some extent be influenced by the amount of remedial treatment received (*4*), data with respect to the number and percent of filled first permanenent molars are also included for a fuller interpretation of the molar mortality rates reported in table 7. These data are shown in Table 7.

Incidence of endemic dental fluorosis (mottled enamel).—The incidence and degree of mottled enamel observed in the groups of children studied are shown in table 8. As in previous studies a child is classified as having endemic dental fluorosis (mottled enamel) when a positive diagnosis of even the mildest type of this affection is made for as few as two teeth. In communities where the fluoride content of the public water supply is in the neighborhood of the minimal threshold of mottled enamel (1.0 p.p.m. of F) the common practice in mottled enamel studies of reporting the incidence as a percentage of children affected (table 8) rather than the percentage of affected teeth, overstates rather than understates the extent of the affection. For instance, at Kewanee (Ill.) where a 12.2 percentage incidence of affection is reported in 123 children examined, a further analysis of the 3,196 permanent teeth, present and in position, of this group shows that approximately 95 percent are free of macroscopic evidence of dental fluorosis. Of the 163 teeth (5.1 percent) diagnosed as positive for dental fluorosis 155 (4.8 percent), were "very mild" and 8 (0.3 percent) were "mild." Distribution of the teeth diagnosed as positive with respect to specific teeth affected showed that 126, or 77 percent, were bicuspids or second molars. As noted in a previous report (*3*) somewhat similar findings were observed at Aurora (Ill.), and as stated in that report such sporadic instances of the mildest forms of dental fluorosis are of no practical esthetic significance.

In accordance with a previously described method of computing a community mottled enamel index (*5*) on the basis of the percentage distribution of clinical severity, the approximate mottled enamel index of Galesburg and Colorado Springs is "slight"; that of East Moline and Kewanee, "border line"; and that of Pueblo, Marion, Lima, Middletown, Quincy, Zanesville, Portsmouth, Elkhart, and Michigan City, "negative."

PUBLIC WATER SUPPLIES

Galesburg, Ill.—For a description of the Galesburg public water supply, see Public Health Reports, *54:* 862–888 (May 26, 1939). No changes in either source or treatment have occurred during the interim between the 1938 study and the present one.

Colorado Springs, Colo.—The Colorado Springs public water supply has been obtained from surface sources for many years. In connection with a mottled enamel survey made in this city in 1935 (Pub. Health Rep., *50:* 1719–1729 (Dec. 6, 1935)) the report noted that the source of the public water supply was melted snow from the south, west, and east slopes of Pike's Peak, and the east and west slopes of Mount Baldy. The water was stored in a system of seven mountain reservoirs located at altitudes ranging from 9.000 to 12,000 feet. From this chain of reservoirs the water was conveyed through a transmission system to settlers at Manitou, thence by gravity to three distribution reservoirs known as the High Line, Mesa No. 1, and Mesa No. 2. These distributing reservoirs were located on a mesa just west of the city and from these reservoirs began the city distribution system and the service mains. Water impounded in both the High Line and the Mesa Reservoirs was obtained from a common source and represented the type of water used by the inhabitants for many years. Years ago Colorado City (that part of the present Colorado Springs lying west of 20th Street, but annexed to Colorado Springs in 1917) was a separate community, comprising according to the 1900 and

TABLE 7.—*Summary of data respecting first molar mortality rates, and information on the number and percent of filled teeth, in selected white children, aged 12–14 years, of 13 cities*

[All teeth referred to in this table are first permanent molars]

	Galesburg, Ill.	Colorado Springs, Colo.	East Moline, Ill.	Kewanee, Ill.	Pueblo, Colo.	Marion, Ohio	Lima, Ohio	Middletown, Ohio	Quincy, Ill.	Zanesville, Ohio	Portsmouth, Ohio	Elkhart, Ind.	Michigan City Ind.
Number of children examined	273	404	152	123	614	263	454	370	330	459	469	278	236
Percent of children with 1 or more missing molars (including extraction indicated)	10.6	3.5	12.5	15.4	14.5	17.5	31.7	36.5	40.9	49.0	38.4	21.9	40.3
Estimated molar population (number of children examined × 4)	1,092	1,616	608	492	2,456	1,052	1,816	1,480	1,320	1,836	1,876	1,112	944
Number of molars showing dental caries experience:													
(a) Filled teeth	170	185	84	57	321	162	457	422	533	502	629	523	444
(b) Teeth with untreated dental caries	234	437	194	172	1,057	515	814	555	360	614	676	399	274
(c+d) Extraction indicated and missing	41	19	24	36	124	66	254	244	235	458	346	95	189
(a+b+c+d) Total	445	641	302	265	1,502	743	1,525	1,221	1,128	1,574	1,651	1,017	907
Percent of molars showing dental caries experience	40.8	39.7	49.7	53.9	61.2	70.6	84.0	82.5	85.5	85.7	88.0	91.5	96.1
Percent of molars with dental caries experience that are filled $\left(\frac{a}{a+b+c+d}\right)$	38.2	28.9	27.8	21.5	21.4	21.8	30.0	34.6	47.3	31.9	38.1	51.4	49.0
First permanent molar mortality, number per 100 children	15.0	4.7	15.8	29.3	20.2	25.1	55.9	65.9	71.2	99.8	73.8	34.2	80.1

1910 Censuses about 12 percent of the total population of the two communities. In its early days Colorado City used water from Sutherland and Bear Creeks in addition to water purchased by contract from Colorado Springs. Dr. Gillett states, however, that as nearly as he can ascertain from some of the old records, Colorado City was using water from a source similar to that of Colorado Springs as far back as 1878. There would seem some justification, therefore, for assuming that the inhabitants of Colorado Springs including the annexed portion, Colorado City, have been using a relatively similar type of water for approximately 60 years. There is, moreover, strong epidemiological evidence that the population of Colorado Springs has been ingesting water with appreciable amounts of fluoride for at least as long as 45 years. A survey made in 1909 (McKay, F. S., in collaboration with Black, G. V.: An Investigation of Mottled Teeth. Dental Cosmos, *58:* 477 (May), 627 (June), 781 (July), 894 (Aug.) 1916) of 927 native born children of this city disclosed an 87.5 percent incidence of mottled enamel. As noted in table 9 of this report, an examination of 404 children in 1940 showed a 73.8 percent incidence of affection, observations that would indicate little difference in the fluoride content of the water used for a decade or more before the first survey and the concentration of fluoride in the water being used at present.

According to information furnished by Dr. O. R. Gillett, health officer of Colorado Springs, the following changes have occurred in the physical setup and source of the water used by the inhabitants of Colorado Springs since 1935. Two dams on the north slope of Pike's Peak were completed, one in 1935, the other in 1937, with a total capacity of 1,133,273,400 gallons. A small settler on French Creek also on the north slope has been completed. Between 25 and 30 percent of the water used at present is obtained from these sources. The transmission line capacity to the city reservoirs has been enlarged and is now about twice the peak load demand. These changes subsequent to 1935 have apparently not influenced the fluoride (F) content of the public water supply. The mean annual fluoride (F) content of 12 monthly samples of the public water supply collected during 1933–34 was 2.5 parts per million. As will be shown later in this paper, 12 monthly samples collected during 1940–41 showed a fluoride (F) content of 2.6 parts per million. The supply is now chlorinated throughout the year.

East Moline, Ill.—The East Moline public water supply is obtained from three deep wells.

Well No. 1 was drilled in 1895 to a depth of 1,340 feet, and was repaired in 1913 and deepened to 1,532 feet. After the installation of well No. 3 in 1937, well No. 1 was held in reserve as a stand-by unit for emergencies; in 1940

TABLE 8.—*Incidence and distribution of endemic dental fluorosis (mottled enamel) in children examined, classified according to the degree of affection*

Macroscopic signs of mottled enamel	Galesburg, Ill.	Colorado Springs, Colo.	East Moline, Ill.	Kewanee, Ill.	Pueblo, Colo.	Marion, Ohio	Lima, Ohio	Middletown, Ohio	Quincy, Ill.	Zanesville, Ohio	Portsmouth, Ohio	Elkhart, Ind.	Michigan City, Ind.
						NUMBER							
Children examined	273	404	152	123	614	263	454	370	330	459	469	278	236
Absent:													
Normal	69	26	56	65	444	151	382	312	307	392	417	254	230
Questionable	74	80	48	43	130	96	62	54	22	60	46	23	6
Present:													
Very mild	110	170	45	13	38	14	10	4	1	7	6	1	0
Mild	17	86	3	2	2	2	0	0	0	0	0	0	0
Moderate	3	36	0	0	0	0	0	0	0	0	0	0	0
Severe	0	6	0	0	0	0	0	0	0	0	0	0	0
						PERCENT							
Total examined	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Absent:													
Normal	25.3	6.4	36.8	52.8	72.3	57.4	84.1	84.3	93.0	85.4	88.9	91.3	97.5
Questionable	27.1	19.8	31.6	35.0	21.2	36.5	13.7	14.6	6.7	13.1	9.8	8.3	2.5
Present:													
Very mild	40.3	42.1	29.6	10.6	6.2	5.3	2.2	1.1	.3	1.5	1.3	.4	0
Mild	6.2	21.3	2.0	1.6	.3	.8	0	0	0	0	0	0	0
Moderate	1.1	8.9	0	0	0	0	0	0	0	0	0	0	0
Severe	0	1.5	0	0	0	0	0	0	0	0	0	0	0
Incidence of affection	47.6	73.8	31.6	12.2	6.5	6.1	2.2	1.1	.3	1.5	1.3	.4	0

well No. 1 was abandoned. Samples of well No. 1 collected in September 1936, and January 1937, showed a fluoride (F) content of 1 part per million.

Well No. 2 was drilled in 1911 to a depth of 1,371 feet. This well was redrilled in 1913 to a depth of 1,850 feet. A sample of water collected in January 1937, showed a fluoride (F) content of 1.6 parts per million.

Well No. 3, located about 50 feet from well No. 1, was drilled in 1937 to a depth of 1,600 feet. The casing is perforated through the St. Peter sandstone (1,000 to 1,060 feet) and water is apparently being obtained from both the St. Peter and the Jordan sandstone (1,495 to 1,585 feet). A sample of water collected July 16, 1937 (Bulletin No. 21, Supplement No. 1, 1938, State Water Survey Division) showed a fluoride (F) content of 0.8 part per million. Water from wells Nos. 2 and 3 is discharged into a new concrete reservoir. Prior to its abandonment in 1940 water from well No. 1 was discharged into the "old" reservoir.

Well No. 4, drilled to a depth of 1,600 feet and drawing water from the Cambrian sandstone, was completed and put into service late in 1940.

The monthly samples collected during the 1935–1936 study (Pub. Health Rep. *52*: 1249 (Sept. 10, 1937)) would indicate that the water used by the population during this period (from wells Nos. 1 and 2) contained about 1.3 parts per million of fluorides (F). As a matter of record, it might also be noted that there is a cross connection between the public water supply and the 60-foot Fairbanks-Morse well.

Kewanee, Ill.—The Kewanee public water supply is obtained from two deep wells into the Cambrian sandstone. Wells into the St. Peter sandstone, which formerly furnished part of the city supply, were abandoned in 1925.

Well No. 1, drilled in 1919, is 2,497 feet in depth. The upper 500 feet of the well is cased with 16-inch pipe and below this casing joined to it by a swedge nipple is 506 feet of 14-inch pipe. Below the 14-inch pipe the well is 12 inches in diameter and is not cased. Well No. 2, drilled in 1927, is 2,438 feet in depth. This well is cased with 20-inch pipe from the surface to 439 feet and with 14-inch pipe from 439 feet to 1,488 feet. Below a depth of 1,488 feet the well is 12 inches in diameter and uncased. The casings do not exclude water from the St. Peter sandstone. A third well, 2,477 feet in depth, was completed in 1940; the mineral composition of the water from this well, however, has no bearing on this study because the clinical examinations were completed prior to its installation.

The Kewanee public water supply is reported to have cross connections with the private water supplies of the Kewanee Boiler Co. and the Walworth Manufacturing Co. for emergency purposes.

Pueblo, Colo.—The public water supply of Pueblo is obtained from surface sources, the Arkansas River. The city of Pueblo has two water systems, that part of the city north of the Arkansas River being supplied by what is known as the Pueblo Water Works, District No. 1 or the North Pueblo water supply, whereas that half of the city located south of the Arkansas River gets its water from another system known as the Pueblo Water Works, District No. 2 or the South Pueblo water supply. Both systems, however, obtain water from the Arkansas River which has been the source of the Pueblo public water supply for more than 50 years. A description of each supply follows:

North Pueblo water supply.—Water is taken from the Arkansas River about 3 miles west of the city and diverted into reservoirs. In 1925 there were six reservoirs and in 1928 one more was added. Treatment consists of preliminary sedimentation, coagulation with aluminum sulfate, followed by sedimentation and disinfection with ammonia-chlorine. Reservoirs Nos. 1, 2, 3, and 4 are used for preliminary sedimentation and reservoirs Nos. 5, 6, and 7 for sedimentation after coagulation. The treatment does not include filtration. Prior to 1928 iron sulfate and lime were used as coagulants, and prior to November 1931 chlorine was used without ammonia.

South Pueblo water supply.—Water is taken from the Arkansas River about 2 miles west of the city and diverted into reservoirs. In 1931 there were four reservoirs and in 1932 three more were added. Treatment consists of preliminary sedimentation, coagulation with aluminum sulfate followed by sedimentation and disinfection with ammonia chlorine. Reservoirs Nos. 1, 2, and 3 are used for preliminary sedimentation and reservoirs Nos. 4, 5, 6, and 7 for sedimentation after coagulation. Under adverse conditions when the water is very turbid, lime and sulfate of iron are used. The treatment does not include filtration. Prior to November 1931, chlorine was used without ammonia.

Marion, Ohio.—The common water supply is obtained from 13 drilled wells located adjacent to the pump house and in the same well field. All of these wells penetrate a limestone deposit which extends within 20 feet of the surface of the ground and is adjacent to the Little Scioto River. The wells vary in diameter from 10 to 14 inches and in depth from 140 to 210 feet.

There have been no changes in the source of this supply during the lifetime of the group of children in this study, but marked changes in the chemical composition of the water probably occurred in 1928.

Until 1928 the supply was untreated but in that year the Marion Water Co. installed a lime-soda softening plant. The treatment is unique in that the holding capacities for the chemical treatment are nearly 24 hours and no filters are used. The plant is operated as follows: Water from the wells is aerated by flowing from a wooden trough over a weir to a splash board. The water then passes to two mixing chambers having a combined detention period of 1.2 hours at 5 m.g.d. (the nominal capacity of the softening plant) and equipped with mechanical stirring devices. Lime, 16 to 20 grains per gallon, and soda ash, 16 to 19 grains per gallon, are applied as the water enters the mixing basin. Occasionally small quantities of alum are used. From the mixing chamber water flows to the clarifier which is equipped for the continuous removal of sludge and has a detention period of 9.4 hours at 5.0 m.g.d. Water is then recarbonated and passes to a settling tank having a detention period of 9.4 hours at 5 m.g.d. The water is then recarbonated a second time after which it is discharged to a clear well from which it is pumped to the distribution system. Marion was one of the cities where it was first noted that the use of lime-soda softening resulted in a reduction of the fluoride concentration of the water.

In our study monthly samples were collected of both the raw water and the treated water. As will be shown later in this paper (table 9), the raw water contained a mean fluoride (F) content of 1.1 parts per million; the treated water, 0.4 part per million.

The hardness of the raw well water averages between 700 and 800 parts per million, that of the treated water in the neighborhood of 200 parts per million. For a year or two after the plant was put into service the water was softened down to less than 100 parts per million, but dropped back to the amount previously indicated (about 200 parts per million) on account of the large expense for the chemicals which, in the company's estimate, could not be justified by the present earning power.

Lima, Ohio.—The water supply of Lima is obtained from surface sources, the Ottawa River, a tributory of the Anglaize, a branch of the Maumee, and is from the Lake Erie watershed. Water is given long storage in the two shallow impounding reservoirs and then filtered through a rapid sand water purification plant.

The water is pumped from the Ottawa River into two storage reservoirs, Lima Lake (400 million gallons capacity) constructed in 1904 and Lost Creek Reservoir (750 million gallons capacity) installed in 1921. The water from these storage reservoirs flows through a 30-inch conduit to two receiving reservoirs located near the pumping station and filtration plant.

The water purification was placed in operation in 1919. Water from the reservoir passes through an over and under baffle mixing chamber having a detention period of 6 minutes at the nominal capacity of the plant (8 m.g.d.). Alum, 1.5 to 3.0 grains per gallon, and sodium silicate, 0.20 to 0.85 grains per gallon, are used as a coagulant (sodium silicate treatment began March 1939). From the mixing chambers the water enters two coagulation basins having a combined detention period of 2 hours at 8 m.g.d. from whence the water passes through rapid sand filters. From the filters water flows to the clear well from where it is pumped to the distribution system. Postchlorination with liquid chlorine is provided at all times and activated carbon is applied when necessary.

This surface water supply has been augmented by ground water from wells during periods of extreme drought, as follows:

(*a*) In 1925 four of the "Tony's Nose" wells (a portion of the formerly abandoned supply) supplied 20 percent of the water consumed for a period of 6 months, or approximately 10 percent of the annual supply for that year.

(*b*) In the winter of 1930–31 the "Tony's Nose" wells supplied approximately 25 percent of the total supply for a 6-month period. The two new wells which were drilled near the Lima Lake Reservoir were also used during 1931. All existing supplies became inadequate in January of 1931 and it became necessary to obtain additional water from four private wells located within the city. It is estimated that during the winter of 1930–31 approximately 60 percent of the water supply was obtained from the various wells in use.

(*c*) In 1934 both the "Tony's Nose" wells and the "Lima Lake" wells were pumped from May to November. Approximately 12 percent of the annual supply was obtained from ground water sources during 1934.

(*d*) In 1936 the "Lima Lake" wells again supplied approximately 5 percent of the annual consumption in that year. Four wells at "Tony's Nose" are connected to the suction of a motor-driven centrifugal pump discharging into the line connecting Lima Lake with the receiving reservoirs. These wells were drilled some time between 1894 and 1900, were abandoned when Lost Creek Reservoir was constructed between 1918 and 1921, and have again been used at times of depleted water supply. Their estimated yield is approximately 1½ million gallons daily, but the water is undesirable on account of its gas and hardness.

In 1930, after the storage reservoirs had been nearly depleted because of deficient rainfall, two deep wells were drilled on the east side of Lima Lake, discharging into that reservoir. The estimated combined yield of these two wells is about 1 million gallons daily but because of the hydrogen sulfide content and the fact that this water materially increases the hardness of the general supply, the use of these wells is held to a minimum.

Middletown, Ohio.—The city of Middletown has obtained its water supply from drilled wells in the valley of Miami River for the past 25 years or more. Except for new wells added from time to time in the same general well field, no changes in the water supply have occurred. In 1924 the water supply of Middletown was obtained from twelve 6-inch and two 12-inch drilled wells. All of these wells are 35 feet deep and obtain water from a gravel and sand deposit which extends practically to the surface of the ground. In 1925 three wells having a diameter of 38 inches were installed in this same well field. Two of the large diameter wells are 165 feet deep and penetrate a gravel and sand deposit which underlies the deposit from which the small diameter wells obtain their supply. The third large diameter well is 40 feet deep and obtains water from the upper gravel stratum. From a chemical standpoint water from the two strata is practically identical. No changes have occurred in the water supply since 1925. Chlorination of the water supply was instituted in 1936 as a general factor of safety. The water receives no other teatment.

Quincy, Ill.—For a description of the Quincy public water supply, see Public Health Reports, *54*: 862–888 (May 26, 1939). No pertinent changes in either source or treatment have occurred during the interim between the 1938 survey and the present study.

Zanesville, Ohio.—Since 1918 the Zanesville public water supply has been obtained from drilled wells located in a 40-acre area situated in the flood plains of and adjacent to the Muskingum River. The original installation consisted of 20 wells, but in 1925 wells Nos. 19 and 20 were abandoned and between that date and 1930 wells Nos. 1 to 18 supplied the city. In 1930, wells Nos. 1 to 11, inclusive were replaced by 11 new wells located in the same well field. The city at the time of this study (January 1940) was supplied by new wells Nos. 1 to 11 and the original wells Nos. 12 to 18.

The wells, varying in diameter from 10 to 13 inches, are all approximately 85 feet deep, and are cased to their entire depth. Water enters the casing through perforations located in that portion of the casing which extends through the water-bearing stratum. Water is obtained from a gravel and sand deposit extending throughout the entire well field which varies in thickness from 20 to 50 feet. This deposit lies below a layer of impervious clay having a minimum depth of 10 feet and a maximum depth of approximately 40 feet. All the water is pumped from the well field to a receiving reservoir by air lift. The water receives no treatment.

Portsmouth, Ohio.—The Portsmouth public water supply is obtained from the Ohio River. The supply is treated in a water purification plant placed in service in 1914. Various improvements and additions have been installed since that date, none of the principal treatment units, however, having been materially changed. Water is pumped from an intake in the Ohio River through mixing basins which have a retention period of 0.25 hour at a flow of 8 m.g.d. Alum, from 0.75 to 2 grains per gallon, is used as a coagulant. It is also necessary to apply from 0.30 to 0.70 grain per gallon of lime to obtain proper coagulation with the alum. The water then passes through two coagulation basins operated in series. Basins Nos. 1 and 2 have a retention period of 6.5 and 3 hours, respectively, based on a flow of 8 m.g.d. From the coagulation basins the water passes through rapid sand filters and then to a clear well from whence it is pumped to the distribution system. Postchlorination with liquid chlorine is applied at all times.

Elkhart, Ind.—The public water supply of Elkhart is obtained from various wells, the description of which follows:

1. Plant wells

Year of installation	*Diameter*	*Depth*
1897	39 ft.	31 ft. 4 in.
1899	38 ft. 6 in.	30 ft. 6 in.
1901	41 ft.	28 ft.

These three wells have been in constant service since their respective dates of installation.

2. Four gravel wall wells 24 inches in diameter and 70 feet deep were installed in 1927. Water from these four wells is pumped into a sand trap; thence the water flows by gravity directly into the reservoir.

3. The Bucklin well, installed in 1891, is 28 feet 4 inches in diameter and 31 feet 5 inches deep. Water from the Bucklin well, and also water from the dug wells (plant wells), is pumped directly into the distribution system.

4. Some water is also obtained during the peak-load season from a well installed in 1924 and made by drilling inside of a dug well to a depth of 45 feet, installing a 16-inch casing, and filling the dug well with gravel. The amount of water, however, contributed by this well to the city supply during the year is negligible.

5. The two Bower Street wells were installed in the summer of 1936. They are gravel packed, 20 inches in di-

ameter and 68 and 70 feet deep, respectively. These wells are used to supplement the other sources of supply during peak-load periods, May to September. During operation water from these wells is pumped directly into the distribution system. These wells are not used during the winter months. Since 1936 the Bower Street wells have furnished approximately 20 percent of the water used during the period of peak demand; this is about 5 percent of the total amount of water used throughout the entire year. During the time that the Bower Street wells are in use, the water is fairly well mixed and the monthly samples collected during the year would constitute a mixture of the several sources that constitute the water supply.

General.—To equalize daily fluctuation and hourly pressure a storage reservoir of 1.25 million gallon capacity and an elevated tank in the center of the distribution system of 500,000 gallon capacity were constructed in 1927. The reservoir is kept filled by the four deep wells installed in 1927. As will be seen in table 10, there was practically no change in the fluoride content of the water supply throughout the year.

Michigan City, Ind.—The public water supply of Michigan City is obtained from Lake Michigan. Prior to 1935 water direct from Lake Michigan was supplied to consumers, no treatment except chlorination being used. The complete filtration plant was placed in service late in 1935.

The water supply is obtained from Lake Michigan through two wooden crib intakes located about 3,000 feet from shore. Water is conveyed from the intakes to the suction wells through a 42- and a 24-inch cast-iron inlet pipe.

Raw water, to which ammonia and chlorine have been added, is pumped to a mixing chamber (detention 30 minutes) equipped with Dorr flocculators. Aluminum sulfate and activated carbon are applied to the suction side of the raw water pumps using dry feed equipment. The water then flows to either of two 1 million gallons settling basins providing 3 hours' detention. The settled water is filtered through four rapid sand gravity filters, each having a capacity of 2 m.g.d. Filtered water flows by gravity to a 1.5 MG underground reinforced concrete reservoir. The water is given additional treatment with chlorine before being pumped to the distribution system and a 750,000-gallon elevated tank.

Chemical analyses of the common water supplies.—As was customary in previous studies, samples of the common water supply were collected, generally monthly, for approximately 1 year. The fluoride content of these waters was estimated colorimetrically by means of the zirconium-alizarin reagent (*6*). The results are given in table 9.

Analyses were made of constituents, other than the fluorides, using a sample from each of the water supplies of the cities studied. Results of these chemical analyses are given in table 10.

DISCUSSION

General findings.—In order that the results of this study might be presented as a coherent whole, the general findings of the study of the 8 suburban Chicago communities previously reported (*3*) will be included with the findings of the study of the 13 cities which form the basis of this report. A summary of the basic observations on the permanent teeth of 7,257 selected white urban school children, aged 12 to 14 years, of these 21 cities is shown in table 11 and figure 1.

Probably the outstanding epidemiological characteristic of these data is the striking variation in the intensity of dental caries attack as evidenced by the marked differences in the amount of dental caries experience. Considering the relative homogeneity of these populations, the method of selecting the study groups, and the similarity of diagnostic standards used, it does not seem likely that such differences can be due to other than the mineral composition of the public water supply. Study of the cause or causes of these differences may shed important light upon either the etiology or the means of partially controlling dental caries.

That the inhibitory agent is the fluoride content of the water supply seems highly probable. An inspection of the range of dental caries experience associated with the use of domestic water of different fluoride concentration discloses an inverse relation in general between

TABLE 9.—*Fluoride (F) content of the public water supplies of the cities studied*

All samples collected from a tap in the distribution system having average domestic use unless otherwise specified
Parts per million

	Galesburg, Ill.	Colorado Springs, Colo.	East Moline, Ill.	Kewanee, Ill.	Pueblo, Colo.		Marion, Ohio		Lima, Ohio	Middletown, Ohio	Quincy, Ill.	Zanesville, Ohio	Portsmouth, Ohio	Elkhart, Ind.	Michigan City, Ind.
					North supply	South supply	Raw	Treated							
1939															
December				[1]0.9 [2].9											
1940															
January								0.5	0.3	0.2		0.2	0.2	0.1	0.1
February								.4	.2	.1		.2	.2		
March		2.8			0.7	0.7	1.0	.4	.4	.2		.2	.1	.1	.1
April		2.6			.7	.8	1.1	.4	.3	.3	0.1	.1	.1	.1	.1
May		2.6			.6	.5	1.1	.6	.2	.2	.1	.2	.2	.1	.1
June		2.4		.9	.4	.5	1.1	.4	.3	.2	.1	.2	.1	.1	.0
July		2.5			.5	.6	1.2	.4	.4	.3	.1	.2	.1	.1	.0
August		2.4			.5	.7	1.1	.4	.3	.2	.1	.2	.1	.1	.1
September		2.5			.6	.6	1.1	.4	.4	.2	.1	.2	.1		.1
October		2.5	1.3		.5	.5	1.1	.4	.4	.2	.1	.2	.1	.2	.1
November		2.6			.6	.5	1.1	.4	.3	.2	.1	.2	.1	.1	.1
December		([3])	1.3		.6	.6	1.1	.4	.3	.2	.2	.2	.1	.1	.1
1941															
January		2.6			.6	.7					.1			.1	.1
February	1.9	2.6			.6	.6					.2			.1	.1
March			1.0								.2				
Mean	[4][5]1.9	[5]2.55	1.20	.90	[5].58	.61	1.10	.43	.32	.21	[4].13	.19	.13	.11	.09

[1] Well No. 1.

[2] Well No. 2.

[3] Container broken.

[4] Single samples of the Galesburg and Quincy public water supplies received in December 1938 disclosed a fluoride (F) content of 1.9 and 0.2 p. p. m., respectively.

[5] 12 monthly samples collected between November 1933 and October 1934 (Pub. Health Rep. 50: 1719-1729 (Dec. 6, 1935) showed a mean fluoride (F) content of 1.86, 2.53, and 0.57 p. p. m. for Galesburg, Colorado Springs, and Pueblo (north supply), respectively.

NOTE.—The limit of the sensitivity of the procedure used for the fluoride determinations may be considered as about 0.1 part per million.

TABLE 10.—*Mineral analyses of the common water supplies of the cities studied*

[All samples collected from a tap in the distribution system having average domestic use unless otherwise specified]

[Parts per million]

	Galesburg, Ill.	Colorado Springs, Colo.	East Moline, Ill.	Kewanee, Ill.	Pueblo, Colo.		Marion, Ohio		Lima, Ohio	Middletown, Ohio	Quincy, Ill.	Zanesville, Ohio	Portsmouth, Ohio	Elkhart, Ind.	Michigan City, Ind.
					North supply	South supply	Raw	Treated							
Residue on evaporation	1,094.4	46.4	1,055.2	1,908.0	524.8	514.4	1,052.8	754.4	372.0	348.0	132.0	485.2	154.4	248.8	196.8
Loss on ignition	40.0	7.2	48.8	86.0	56.0	74.4	106.4	32.0	106.0	45.0	14.0	92.0	35.2	36.8	49.6
Fixed residue	1,054.4	39.2	1,006.4	1,822.0	468.8	440.0	946.4	722.4	266.0	303.0	118.0	393.2	119.2	212.0	147.2
Silica (SiO_2)	7.2	8.0	7.5	38.0	14.4	16.0	28.0	19.6	2.0	8.0	10.0	13.0	8.0	12.0	17.6
Iron (Fe)	.1	.04	.04	.01	.06	.06	.08	.04	.01	.04	0	.05	.01	.01	.02
Aluminum (Al)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Calcium (Ca)	57.2	8.0	64.6	115.7	76.0	78.1	206.8	52.0	60.0	85.0	22.9	87.9	25.2	54.9	36.6
Magnesium (Mg)	25.3	1.6	27.8	38.0	27.2	25.5	55.9	19.2	17.9	28.4	7.4	17.4	4.2	20.1	12.0
Sodium and potassium (calculated as Na)	296.7	2.8	282.0	506.2	41.1	38.8	17.7	145.7	12.1	5.0	3.7	33.2	8.8	4.5	1.7
Carbonate (CO_3)					9.6	8.4									
Bicarbonate (HCO_3)	295.2	23.2	309.9	300.1	154.9	164.7	341.6	42.7	148.8	317.2	37.8	153.7	28.0	245.2	136.6
Sulfate (SO_4)	351.7	4.9	240.3	308.6	213.1	203.8	463.2	450.9	89.7	49.4	45.3	89.3	57.6	16.0	21.4
Nitrate (NO_3)	3.1	1.0	3.9	6.6	5.5	4.8	1.0	1.0	5.5	5.3	4.4	.4	3.4	.4	0.6
Chloride (Cl)	190.5	.5	265.0	689.0	10.5	9.0	4.0	4.0	18.0	6.0	5.0	106.0	8.0	3.0	5.0
Phosphate (PO_4)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fluoride (F)	1.9	2.6	1.3	.9	.7	.7	1.1	.4	.3	.2	.1	.2	.1	.1	.1

The dates of receipt of these samples of water were as follows: Galesburg Feb. 1941, Colorado Springs May 1940, East Moline October 1940, Kewanee December 1939, Pueblo March 1940, Marion April 1940, Lima June 1940, Middletown January 1940, Quincy May 1940, Zanesville October 1939, Portsmouth April 1940, Elkhart November 1940, and Michigan City January 1940.

Assistant Chemist C. G. Remsburg carried out the determinations other than fluoride, using mostly the methods given in the Standard Methods of Water Analysis of the American Public Health Association. The phosphate was determined colorimetrically by an adaptation of the Benedict and Theis method (J. Biol. Chem., 61: 63 (1924)).

TABLE 11.—*Summary of dental caries findings in 7,257 selected white school children, aged 12 to 14 years, in 21 cities of 4 States in relation to the fluoride (F) content of the public water supply*

	Galesburg, Ill.	Colorado Springs, Colo.	Elmhurst, Ill.	Maywood, Ill.	Aurora, Ill.	East Moline, Ill.	Joliet, Ill.	Kewanee, Ill.	Pueblo, Colo.	Elgin, Ill.	Marion, Ohio	Lima, Ohio	Evanston, Ill.	Middletown, Ohio	Quincy, Ill.	Oak Park, Ill.	Zanesville, Ohio	Portsmouth, Ohio	Waukegan, Ill.	Elkhart, Ind.	Michigan City, Ind.
SELECTION OF STUDY GROUPS																					
Total number of 12- to 14-year-old children present at time of selection	918	1,444	633	873	1,625	352	1,412	522	1,412	1,030	1,010	1,411	2,125	1,013	1,063	1,662	1,248	1,228	1,354	942	654
Number of 12- to 14-year-old white children whose histories on repeated questioning indicated continuity of exposure and who were examined	273	404	170	171	633	152	447	123	614	403	263	454	256	370	330	329	459	469	423	278	236
Percentage of the total present who were examined	29.7	28.0	26.9	19.6	39.0	[illegible]	31.7	23.6	43.5	39.1	26.0	32.2	12.0	36.5	31.0	19.8	36.8	38.2	31.2	29.5	36.1
WATER SUPPLY																					
Source [1]	G	S	G	G	G	G	G	G	S	G	G	S	S	G	S	S	G	S	S	G	S
Total hardness in parts per million	247	27	323	75	329	276	349	445	302	103	209	223	131	329	88	132	291	80	134	220	141
Mean fluoride (F) content in parts per million of cities studied	1.9	2.6	1.8	[2] 1.2	1.2	[3] 1.2	1.3	0.9	0.6	0.5	[4] 0.4	0.3	0	0.2	0.1	0	0.2	0.1	0	0.1	0.1
[5]		> 1.4			1.4 to 1.0				0.9 to 0.5							< 0.5					
CLINICAL EXAMINATION																					
Unit basis of measurement:																					
Teeth:																					
Dental caries experience, permanent teeth, per 100 children examined	236	246	252	258	281	303	323	343	412	444	556	652	673	703	706	722	733	772	810	823	1,037
		244				294			416							740					
First permanent molar mortality, per 100 children examined	15.0	4.7	11.8	11.7	14.5	15.8	19.5	29.3	20.2	20.3	25.1	55.9	42.6	65.9	71.2	31.0	96.8	73.8	79.9	34.2	80.1
Tooth surface:																					
Dental caries experience, proximal surfaces, superior permanent incisors, per 100 surfaces	0.46	0.31	0.60	0.89	0.78	0.16	1.3	1.4	0.47	4.1	3.3	3.2	10.7	7.1	11.2	9.0	11.4	10.4	17.7	11.2	18.0
Child:																					
Percent of children with 1 or more permanent teeth showing dental caries experience	72.2	71.5	74.7	70.2	76.5	79.6	81.7	82.1	89.4	88.6	94.3	97.8	96.1	98.1	97.6	95.7	97.4	98.7	96.9	98.6	100.0
Percentage incidence of endemic dental fluorosis (mottled enamel)	47.6	73.8	40.0	33.3	15.0	31.6	25.3	12.2	6.5	4.2	6.1	2.2	1.6	1.1	0.3	0.6	1.5	1.3	0.2	0.4	0

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[1] S=surface water; G=ground water.

[2] There is both presumptive and direct evidence that prior to a few years ago the Maywood water contained probably 1.4 to 1.6 p.p.m. of F (Pub. Health Rep. 56: 761–792 (Apr. 11, 1941)).

[3] There is both presumptive and direct evidence that prior to a few years ago the East Moline water contained as much as 1.5 p.p.m. of F (Pub. Health Rep. 52: 1249–1264 (Sept. 10, 1937)).

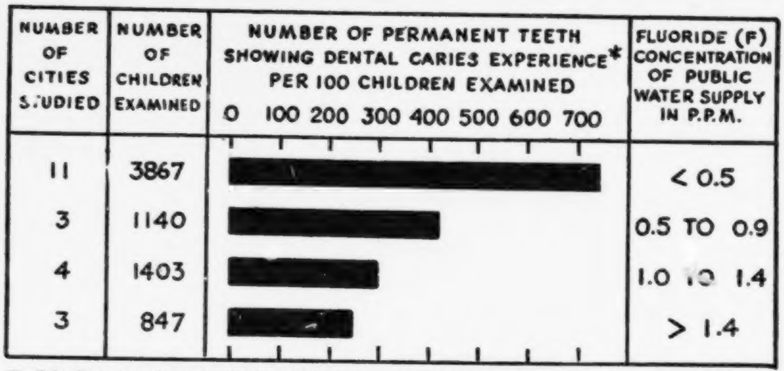
[4] Prior to 1928 this water supply probably contained about 1.1 p. p. m. of F. See reference in text concerning treatment of raw water; see table 9 for its analysis.

[5] Range of fluoride (F) concentration of water supply and dental caries experience rate of children in these specified groupings.

the amount of dental caries and the fluoride concentration of the common water supply. Relatively low dental caries experience rates are found associated with the use of domestic waters whose fluoride (F) concentrations have a range of 1 or more parts per million. Intermediately, e.g., at concentrations of 0.9 to 0.5 part per million, the influence is less marked than at the higher concentrations; nevertheless, the dental caries experience rates are distinctly lower than those associated with the use of relatively fluoride-free waters. A further inspection of the data reported in table 11 and figure 2 for those cities whose public water supplies contain less than 0.5 part per million discloses a considerable variation among those cities characterized by high dental caries experience. This variation is marked, particularly between those communities whose public water supplies did not show fluoride (F) in excess of 0.2 part per million. As has been pointed out, however, the limit of sensitivity of the method of determination may be considered as about 0.1 part per million and hence further discussion at present of this variation would not seem justified. For this reason no attempt was made at this time to fit a curve to the data shown in figure 2.

A correlation between the dental caries experience rates and the mean fluoride (F) content of the public water supply of each of the 21 cities studied is shown in figure 2.

AMOUNT OF DENTAL CARIES (PERMANENT TEETH) OBSERVED IN 7257 SELECTED 12-14 YEAR OLD WHITE SCHOOL CHILDREN OF 21 CITIES OF 4 STATES CLASSIFIED ACCORDING TO THE FLUORIDE CONCENTRATION OF THE PUBLIC WATER SUPPLY.



* DENTAL CARIES EXPERIENCE IS COMPUTED BY TOTALING THE NUMBER OF FILLED TEETH (PAST DENTAL CARIES), THE NUMBER OF TEETH WITH UNTREATED DENTAL CARIES, THE NUMBER OF TEETH INDICATED FOR EXTRACTION, AND THE NUMBER OF TEETH MISSING (PRESUMABLY BECAUSE OF DENTAL CARIES).

FIGURE 1

RELATION BETWEEN THE AMOUNT OF DENTAL CARIES (PERMANENT TEETH) OBSERVED IN 7257 SELECTED 12-14 YEAR OLD WHITE SCHOOL CHILDREN OF 21 CITIES OF 4 STATES AND THE FLUORIDE (F) CONTENT OF PUBLIC WATER SUPPLY

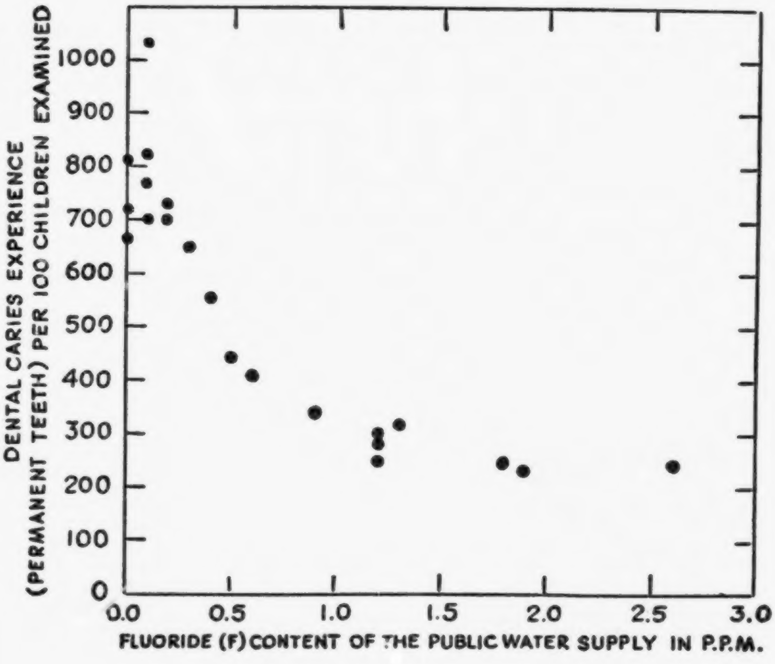


FIGURE 2

SUMMARY

1. A study of the intensity of dental caries attack, as evidenced by the observed dental caries experience, disclosed striking differences among children of different cities. This study embraced 7,257 white urban school children, aged 12 to 14 years, of 21 cities; in the main the children were apparently of largely comparable circumstances and the groups examined were relatively equitable respecting sex ratio. These totals, 7,257 children of 21 cities, represent the 4,425 children of 13 cities reported in detail in this paper and the 2,832 children of 8 suburban Chicago communities previously reported (3). See table 11 and figures 1 and 2 of this report. The groups studied were limited to those children *continuously exposed throughout life to the variable under investigation* (the common water supply). Clinical examinations in all 21 cities were made by the same two dental officers and in each city an equal number of children were examined by each examiner. It seems unlikely that such

marked differences in the prevalence of dental caries can be explained on the basis of the hardness of the domestic water, the hours of sunshine, or gross dissimilarities in diet (water excluded).

2. A general inverse correlation between the fluoride concentrations of the public water supplies in the 21 cities studied and the amount of dental caries was observed. Differences in dental caries experience rates of as much as two and three times the observed minimal were not unusual; the highest rate, 1,037, at Michigan City (Ind.) was 4.4 times that observed in the city with the lowest rate, 236, at Galesburg (Ill.). Strikingly low dental caries prevalence was found associated with the continuous use of domestic waters whose fluoride (F) content was as low as about 1 part per million, a concentration which under the conditions prevailing in the localities studied produced only sporadic instances of the mildest forms of dental fluorosis of no practical esthetic significance.

3. As in previous studies, marked differences were observed with respect to: (*a*) The amount of dental caries experience in the proximal surfaces of the four superior permanent incisors, and (*b*) the first permanent molar mortality rates. Of the 4,425 children of the 13 cities whose caries experience is reported in detail in this report, the 2,859 children living in communities whose public water supply contained less than 0.5 p.p.m. of fluoride (F) showed about 19 times as much proximal surface caries experience in the four superior permanent incisors as was observed in the 1,566 children living in cities where the common water supplies contained from 0.6 to 2.6 p.p.m. of fluoride (F). In these same two groups of children, the first permanent molar mortality rate for those living where the water supply contained less than 0.5 p.p.m. of fluoride (F) was about 4 times as high as that observed in the children using a domestic water containing more than 0.5 p.p.m. of fluoride (F) (66.0 and 15.6 per 100 children examined, respectively). Inasmuch as the group with the higher first permanent molar mortality rate showed 38 percent of its total first permanent molar caries experience with fillings as opposed to only 26 percent in the group characterized by the lower mortality rate, there would seem justification in assuming that such differences in first permanent molar mortality rates are influenced to a considerable degree by a variation in either the intensity of dental caries attack, and/or the resistance of the teeth to caries attack.

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DOMESTIC WATER AND DENTAL CARIES VI. The Relation of Fluoride Domestic Waters to Permanent Tooth Eruption

EDWIN M. SHORT

Various observations (*1, 2, 3*) have been made indicating an apparent delay in tooth eruption in individuals living in areas of endemic dental fluorosis; other investigators (*4, 5*) have failed to confirm these findings. These observations, however, have dealt largely with areas of high endemicity, the concentration of fluorides in the drinking waters being relatively high. It has been reported (*6*) that the feeding of high concentrations of fluorides to rats has substantially reduced the rate of eruption of the lower incisor teeth. As a result of recent field studies concerning the effect of smaller concentrations of fluorides on dental caries (*7, 8*), certain data have been made available permitting the investigation of evidence concerning any delay in permanent tooth eruption in areas of lower fluoride concentration. Because of the inverse relationship between endemic dental fluorosis and dental caries prevalence, it seemed advisable to determine, if possible, whether this variable might disclose an appreciable difference in years of post eruptive tooth exposure to the risk of dental caries.

The eight Chicago suburban communities (7) besides presenting unusual opportunities to study dental caries, also offer certain advantages in the study of comparative eruption rates. These advantages include homegeneity of populations studied, similarity of climatological factors, and range in fluoride (F) content of the public water supplies, 0.0–1.8 parts per million. All dental examinations were made by one of two examiners

(Assistant Dental Surgeons (R) David C. Johnston and Edwin M. Short) with approximately an equal number in each city being done by each examiner. In addition to these eight communities, Galesburg, Ill., with a water supply containing approximately the same concentration of fluorine as Elmhurst, the highest fluoride community in the Chicago area study, is included in this series along with the control city of Quincy, which uses fluoride-free Mississippi River water. In order to determine the effect, if any, on tooth eruption of a public water supply containing more fluorine than any of those studied in Illinois, Colorado Springs, Colo., the water supply of which contains the highest concentration of fluorine of any city surveyed by the same examiners, is also included in this series of cities. In order to provide a measure of control against this high fluoride community, the nearby city of Pueblo, whose water supply is relatively low in fluoride content, is also included.

In this series of 12 cities, 4 (Quincy, Evanston, Oak Park, and Waukegan) have water supplies that are virtually fluoride free; 2 (Elgin and Pueblo) use water containing approximately 0.5 p.p.m. of fluoride (F); the water supplies of 3 communities (Joliet, Maywood, and Aurora) contain slightly over 1 p.p.m., and those of 2 more (Galesburg and Elmhurst) have just below 2 p.p.m. The highest fluoride city in this series, namely Colorado Springs, uses a water supply the fluoride (F) content of which is 2.6 p.p.m. An inverse relationship between fluoride content of the public water supply and dental caries prevalence rates has been demonstrated in these 12 communities (*8*); those cities having more than 1 p.p.m. of fluoride (F) in their water supplies show from one-half to one-third the caries experience rates of the fluoride free cities. Those two communities using water of approximately 0.5 p.p.m. of fluoride (F) lie approximately midway between the higher fluoride and fluoride free communities in caries experience rates.

On the basis of a previously described method of computing a community mottled enameled index (*9*), 3 of these 12 cities (Colorado Springs, Galesburg, and Elmhurst) would be classified as "slight" and 3 (Joliet, Maywood, and Aurora) as "borderline." The six remaining communities show a "negative" mottled enamel index. Those communities whose public water supplies contain fluorine in concentrations between 1 and 2 p.p.m. show a very mild type of dental fluorosis characterized by a few small white opaque areas on a small percentage of the permanent teeth.

In the dental examinations as conducted in the 4,453 children, age 12 to 14 years, of these 12 communities, definite information was recorded for each tooth, erupted or unerupted. Erupted teeth were marked as normal or carious, i.e., untreated, filled, missing, or indicated for extraction. Only those teeth that had erupted to the point of being in occlusion or in line of occlusion were classified as erupted. Unerupted teeth were marked as such. Intermediately is the partially erupted tooth, a tooth being placed in this classification from the time the point of the cusp makes its appearance through the mucosa until it may be classified as fully erupted.

DATA

Table I shows the mean number of erupted and partially erupted teeth in each of the 12 communities studied. They are placed in the order of decreasing fluoride content of the public water supply.

In tables II, III, and IV the cities have been grouped according to the fluoride content of the public water supply. Group 1 consists of Colorado Springs, using a domestic water containing 2.6 p.p.m. of fluoride (F). Group 2, consisting of Galesburg and Elmhurst, use public water supplies of 1.9–1.8 p.p.m. fluoride (F) content. In group 3 are Joliet, Maywood, and Aurora, whose water supplies contain fluoride (F) in concentrations of 1.3–1.2 p.p.m. Group 4 is made up of Pueblo and Elgin with 0.6–0.5 p.p.m. of fluoride (F) in their drinking waters. Quincy, Evanston, Oak Park, and Waukegan make up group 5; the water supplies of these cities have 0.1–0.0 p.p.m. of fluoride (F), a concentration which may be considered "fluoride-free." The distribution of the children by age and sex within each group is shown according to the total number of erupted permanent teeth.

Table V is used as the basis of a comparison of the group of 0.0–0.1 p.p.m. (F) communities (No. 5) with each of the other groups (Nos. 1, 2, 3, and 4). The

TABLE I.—*Summary of permanent tooth eruption data, third molars excluded, of selected 12–14 year old white school children of 12 communities in Illinois and Colorado*

CITY	NUMBER OF CHILDREN EXAMINED			ERUPTED PERMANENT TEETH* (a)	PARTIALLY ERUPTED PERMANENT TEETH (b)	TOTAL (a + b)
	M	F	Both			
				(A) Number		
Colorado Springs, Colo. (2.6)**	183	221	404	10190	235	10425
Galesburg, Ill. (1.9)	137	136	273	6987	162	7149
Elmhurst, Ill. (1.8)	89	81	170	4414	90	4504
Joliet, Ill. (1.3)	196	251	447	11984	132	12116
Maywood, Ill. (1.2)	92	79	171	4462	81	4543
Aurora, Ill. (1.2)	346	287	633	16559	253	16812
Pueblo, Colo. (0.6)	314	300	614	16088	259	16347
Elgin, Ill. (0.5)	199	204	403	10605	170	10775
Quincy, Ill. (0.1)	142	188	330	8725	146	8871
Evanston, Ill. (0.0)	111	145	256	6683	122	6805
Oak Park, Ill. (0.0)	138	171	329	8634	159	8793
Waukegan, Ill. (0.0)	190	233	423	11085	214	11299
				(B) Number per Child		
Colorado Springs, Colo.				25.22	0.58	25.80
Galesburg, Ill.				25.59	0.59	26.19
Elmhurst, Ill.				25.96	0.53	26.49
Joliet, Ill.				26.81	0.30	27.11
Maywood, Ill.				26.09	0.47	26.57
Aurora, Ill.				26.16	0.40	26.56
Pueblo, Colo.				26.20	0.42	26.62
Elgin, Ill.				26.32	0.42	26.74
Quincy, Ill.				26.44	0.44	26.88
Evanston, Ill.				26.11	0.48	26.58
Oak Park, Ill.				26.24	0.48	26.73
Waukegan, Ill.				26.21	0.51	26.71

* Permanent teeth which have been extracted are counted as erupted.

** Figure in parenthesis shows fluoride (F) content of public water supply in parts per million.

TABLE II.—*Distribution of 2150 white school boys, age 12-14 years, according to number of erupted permanent teeth. Children grouped on basis of fluoride (F) content of their public water supply*

NO. OF ERUPTED PERMANENT TEETH	12 YEARS					13 YEARS					14 YEARS				
	2.6*	1.8-1.9	1.2-1.3	0.5-0.6	0.0-0.1	2.6	1.8-1.9	1.2-1.3	0.5-0.6	0.0-0.1	2.6	1.8-1.9	1.2-1.3	0.5-0.6	0.0-0.1
28	12	18	78	41	55	25	41	117	97	116	46	42	147	135	145
27	5	3	16	14	15	5	8	37	22	24	6	11	21	13	23
26	7	8	15	18	18	6	9	18	31	26	3	5	11	6	16
25	4	6	18	10	13	3	4	7	5	12	2	3	6	3	5
24	5	12	17	15	21	6	5	14	8	15	3	4	4	5	4
23	1	3	14	10	7	2	1	4	6	5		2	1	1	2
22	1	8	13	7	16		1	2	2	1			2	2	1
21	6	4	6	8	12	2	2	3	5	1			1	1	2
20	1	2	7	8	2	3	1	2	3	4			1	1	
19	2	4	7	11	6		1	4	1	1					
18	6		9	6	5		1	1	3	2					
17	3	2	5	5	2	1						2	1		
16	1	4	3	2	1		2	4	1	1					
15	3	2	4	1	3	1		1	2						
14		3	4		2	2		1		2					1
13	2	1	2	1	3			2							
12	8		3	1	3		1	1							
11				1											
10				1	1										
Total	67	80	221	160	185	56	77	218	186	210	60	69	195	167	199
Mean no. of erupted teeth per child	21.55	23.24	24.18	23.82	24.10	25.32	26.05	26.23	26.34	26.52	27.50	26.87	27.39	27.49	27.35

* Fluoride (F) content of public water supply in parts per million.

TABLE III—*Distribution of 2303 white school girls, age 12–14 years, according to number of erupted permanent teeth. Children grouped on basis of fluoride (F) content of their public water supply*

NUMBER OF ERUPTED PERMANENT TEETH	12 YEARS					13 YEARS					14 YEARS				
	2.6*	1.8-1.9	1.2-1.3	0.5-0.6	0.0-0.1	2.6	1.8-1.9	1.2-1.3	0.5-0.6	0.0-0.1	2.6	1.8-1.9	1.2-1.3	0.5-0.6	0.0-0.1
28	20	27	76	83	83	48	44	161	102	161	45	49	180	117	154
27	5	5	19	21	42	14	9	26	23	42	8	4	18	15	37
26	10	6	12	22	23	7	9	13	16	23	8	3	12	2	16
25	1	4	15	16	19	5	2	7	8	17	2	3	6	1	7
24	9	15	11	13	21	1	8	5	3	10			4	2	5
23	6	4	5	7	12	1	4	4	5	3	1	1		2	2
22	1	3	8	11	12		1	4	7	8			1	1	1
21	2	1	6	7	9	2	1	2	2	4					1
20	3	2	5	1	4	1	2		2	2					
19	4	2	5	3	3							1			
18	1	3	1	3	5				2	2					
17	5	1	2	2	5	1	1		2				1		
16	1				2	1		2	2						
15					5		1								
14			2		2		1								1
13	1		1	1											
12	7				1										
Total	76	73	168	190	248	81	83	227	174	272	64	61	222	140	224
Mean no. of erupted teeth per child	23.93	25.14	25.58	25.81	25.09	26.80	26.34	27.23	26.54	26.88	27.45	27.46	27.58	27.49	27.34

* Fluoride (F) Content of public water supply in parts per million.

Table IV.—*Distribution of 4453 white school children, age 12–14 years, according to number of erupted permanent teeth*

Number of erupted permanent teeth	I 2.6 ppm. F		II 1.8–1.9 ppm. F		III 1.2–1.3 ppm. F		IV 0.5–0.6 ppm. F		V 0.0–0.1 ppm. F	
	M	F	M	F	M	F	M	F	M	F
28	83	113	101	120	342	420	273	302	316	398
27	16	27	22	18	74	63	49	59	62	121
26	16	25	22	18	44	37	55	40	60	62
25	9	8	13	9	31	28	18	25	30	43
24	14	10	21	23	35	20	28	18	40	36
23	3	8	6	9	19	9	17	14	14	17
22	1	1	9	4	17	13	11	19	18	21
21	8	4	6	2	10	8	14	9	15	14
20	4	4	3	4	10	5	12	3	6	6
19	2	4	5	3	11	5	12	3	7	3
18	6	1	1	3	10	1	9	5	7	7
17	4	6	4	2	6	3	5	4	2	5
16	1	2	6		7	2	3	2	2	2
15	4		2	1	5		3		3	5
14	2		3	1	5	2			5	3
13	2	1	1		4	1	1	1	3	
12	8	7	1		4		1		3	1
11							1			
10							1		1	
Total....	183	221	226	217	634	617	513	504	594	744
Mean no. of erupted teeth per child	24.66	25.69	25.31	26.18	25.87	26.91	25.93	26.58	26.04	26.42

comparisons are made by single age for both sexes. The chi-square (X^2) values are computed for each of the comparisons made, permitting the determination of P. which represents the probability that the differences observed are likely to be due to chance.

DISCUSSION

When the children of Colorado Springs, or group 1 (table V) are compared with group 5, composed of children from cities using fluoride free waters, a significant difference is disclosed in the eruption rates of the permanent teeth for the 12-year-old age group of both sexes. Thus, children 12 years of age in the higher fluoride cities show an appreciably lower eruption rate than those in the fluoride free group. It should be pointed out that in group I, the distribution of 12 year old children according to the number of erupted permanent teeth shows an unusually large number with 12 erupted teeth. When all children with 12 or less erupted teeth are eliminated from both groups, a comparison of the 12 year olds of group I with those in group V shows a significant difference for the boys (P=0.0445) but not for the girls (P=0.2050). A similar condition prevails to a lesser degree in the 13-year-old boys but the 14-year-olds, both sexes, show no significant difference.

Group 2 consisting of the next highest fluoride (F) cities (1.8–1.9) p.p.m. in this series, shows no significant difference from group 5 in eruption rates, although the 12 and 13 year old boys of group 2 are slightly behind the boys of the same age in group 5. The next 2 groups of cities, Nos. 3 and 4, using water supplies with fluoride (F) contents of 1.2–1.3 and 0.5–0.6 p.m.m., respectively, show no evidence of a significant delay in eruption at any of the ages examined; in fact, at some ages in each group there is a tendency toward the opposite effect of a slightly higher eruption rate.

The basic purpose in analyzing the data from these population groups was to determine whether or not appreciable difference existed in the time of eruption of the permanent teeth. If for instance, significant differences were found to exist between those characterized by low dental caries prevalence in contrast to those having a high dental caries experience, the influence of this time variable respecting risk of exposure to dental caries attack would necessitate careful appraisal. But, as has been shown above (tables II, III, and IV), the marked differences in dental caries experience (*8*) between populations using fluoride-free domestic waters and those using waters containing as much as 1.2–1.3 parts per million of fluoride (F) cannot be explained on the basis of differences in the time of eruption.

The disclosure of a significant difference in the eruption rate recorded for the Colorado Springs population (2.6 p.p.m. of F in the public water supply) seemingly warrants further comment in the light of its possible relation to other closely related physiological fields. The unusual effects of trace elements on physiological processes has opened a vast field for scientific investigation. Moulton (*11*) referring to dental fluorosis has recently stated that "It is a new and quite different illustration of the fact that very minute quantities of certain elements and of certain organic compounds (vitamins and hormones) sometime have extraordinary effects upon living organisms." More recently Shils and McCollum (*12*) state that like vitamins, the indispensable "traces" appear to be keys to fundamental physiologic processes little understood and a challenge to nutritionists and physiologists. The investigation of the physiological effects of one trace element, fluoride, by the epidemiological method has recently been described by Dean (*13*)

Table V.—*Comparison of 5 groups of children, age 12 to 14 years, classified on basis of fluoride content of public water supply, showing chi-square values and probability of results occurring by chance*

Groups compared**	Value of X^2 and P according to age and sex of children*											
	12 Years				13 Years				14 Years			
	Males		Females		Males		Females		Males		Females	
	X^2	P	X^2	P	X^2	P	X^2	P	X^2	P	X^2	P
1 vs. 5	16.84	0.0021	13.26	0.0042	7.81	0.0698	0.39	0.8465	0.34	0.5598	0.06	0.8065
2 vs. 5	4.07	0.3977	3.56	0.3187	3.04	0.3864	2.95	0.2304	3.50	0.0614	3.14	0.7843
3 vs. 5	2.23	0.6950	1.65	0.6525	3.94	0.2693	4.10	0.1300	0.33	0.5657	9.02	0.0027
4 vs. 5	5.02	0.2855	4.06	0.2561	3.71	0.2992	2.57	0.2854	3.21	0.0736	9.95	0.0016

* For purposes of calculating X^2 the following values of N^1 were used: 12 Years Males $N^1 = 5$, females $N^1 = 4$; 13 Years Males $N^1 = 4$, females $N^1 = 3$; 14 Years Males $N^1 = 2$, females $N = 2$.

** Group 1 = 2.6 ppm. fluoride (F); group 2 = 1.8 – 1.9 ppm; group 3 = 1.2 – 1.3 ppm; group 4 = 0.5 – 0.6 ppm.; group 5 = 0.0 – 0.1 ppm.

who also states (*14*) that for certain physiological problems, particularly in the field of pharmacology and nutrition, the application of quantitative epidemiological methods may be the means of bringing to light physiological secrets latent in large population groups. Acceleration or retardation of permanent tooth eruption is a matter of interest to both anthropologists and those concerned with growth and development. Racial differences in eruption time have been reported by Steggerda and Hill (*10*), who state that in the four racial groups studied, the early eruption time of teeth to be in the following order: Navajo, Negro, Maya, and White. In the data reported in the present paper the genetic constitution and environmental conditions (fluoride intake excluded) would seem largely comparable in the 12 white school populations studied in Illinois and Colorado. The only variable recognizable at present which may account for the significant difference noted at Colorado Springs would seem the higher fluoride intake. The suggestion that one growth index (rate of permanent tooth eruption) might be influenced by a minute fluoride intake raises the question of the possible influence of this variable in studies, other than dental, in closely coordinative physiological fields.

Although it is possible that the difference in eruption between the high fluoride and fluoride-free cities may be due to the lower dental caries experience rates in the high fluoride areas resulting in a longer retention of the deciduous teeth, this seems improbable, as the dental caries prevalence rates in these high fluoride areas are comparable to those found in the more moderate fluoride areas which show no lower eruption rates than the fluoride-free cities.

SUMMARY

1. Public water supplies containing fluorides in concentrations of 2.6 parts per million appear to be associated with delayed permanent tooth eruption in children 12 years of age who have been continuously exposed to the water supply. Significant differences were not observed in those populations using waters containing less than 2.0 parts per million of fluoride (F).

2. The marked difference in dental caries prevalence between populations using fluoride-free domestic waters and those using as much as 1.2–1.3 parts per million of fluorides (F) cannot be explained on the basis of differences in time of permanent tooth eruption.

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DENTAL CARIES EXPERIENCE IN RELOCATED CHILDREN EXPOSED TO WATER CONTAINING FLUORINE. I. Incidence of New Caries After 2 Years of Exposure Among Previously Caries-Free Permanent Teeth

By HENRY KLEIN

In the course of systematic dental examination of persons of Japanese ancestry residing in War Relocation Authority centers, two groups of children at two different centers were examined in the summer of 1943 and again in the summer of 1945. Early in 1942 both groups, because of their Japanese ancestry, had been transferred with their parents from homes in Los Angeles and environs to an assembly center near Los Angeles. In the autumn of 1942 they were again transferred, 120 to a center in California and 196 to Arizona.

The children relocated to the California center consumed fluoride-free water originating from melted snows coming off a precipitous mountain rising to a height of more than 14,000 feet, less than 20 miles from the residence area. Analysis of this water revealed a fluorine content of 0.1 p.p.m., a value within the error of measurement. The children relocated to the Arizona center consumed water originating from two deep wells drilled through the desert floor to a depth of approximately 400 feet. This water contained fluorine to the extent of 3 p.p.m. Water from the central source was piped to each family apartment in both centers.

Because of the relatively high fluorine content of the water in the Arizona center, an attempt was made by the Relocation Authority to remove the fluorides. Bone-meal filters were installed only at selected water outlets to which the population had to travel to obtain fluoride-free drinking water. After a trial of several months, treatment of the water in this manner was discontinued. Bottled fluorine-free waters were shipped into the center and sold to residents who reserved such water chiefly for the preparation of dietary formulae for infants. The children of school age obtained their drinking water from the nearest tap, which provided water containing fluorine (except during the 3-month period mentioned above, when fluorine-free water could be obtained, if so desired, at several selected outlets).

In the early summer of 1943, the school children were examined with the aid of dental mirrors and explorers; the same children were reexamined 2 years later in the summer of 1945. All examinations were recorded by the methods previously utilized in the Hagerstown Dental Studies. ("A procedure for the recording and statistical processing of dental examination findings": Klein, Henry, and Palmer, C. E.: J. Dent. Res., *19*: 243 (1940).) During the 2-year interval, the children were restricted to their respective centers, since movement in and out was controlled by military authority. Their diets were quite similar and adequate.

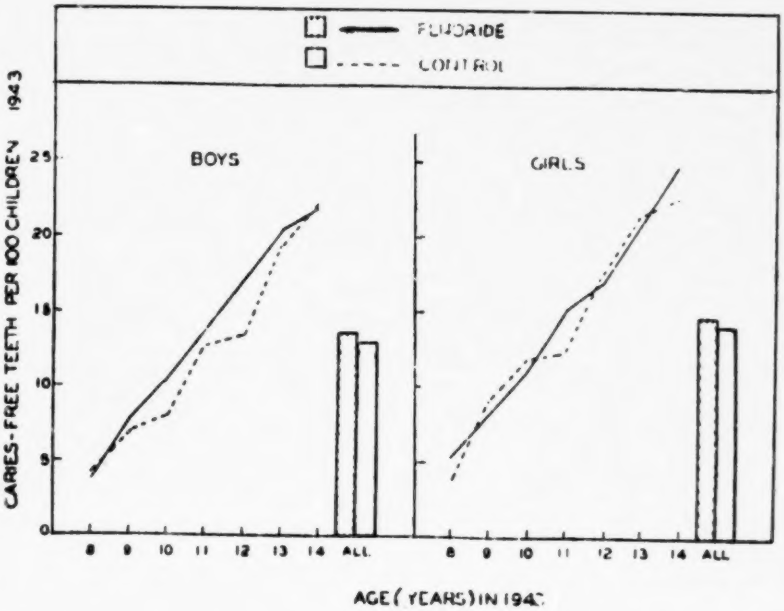

FIGURE 1

Analysis of the dental findings obtained in 1943 and in 1945 reveals that a fluorine content of 3 p.p.m. in the drinking water is associated with a marked reduction in new caries in teeth present in the mouth and free of caries at the beginning of exposure.

FINDINGS

During the 2-year interval between 1943 and 1945, the 196 children were exposed to fluoride water in the Arizona Relocation Center and 120 consumed fluoride-free water in the California center. Distribution by sex and age is shown in table 1.

At the time of the first examination in 1943, the boys as well as the girls of the two areas were quite similar with regard to the number of caries-free permanent teeth present in the mouth. The boys and girls destined to reside in the fluoride area had an average of 13.4 and 14.7 caries-free permanent teeth, respectively, and the boys and girls in the control area, 12.8 and 14.1 (fig. 1).

After a 2-year residence in their respective areas, the two groups of children showed a marked difference in the number of teeth newly attacked by caries (see table 2 and fig. 2). For example, in the fluoride area, boys who were 8 years old in 1943 developed about 22 new DMF (decayed, missing, or filled) teeth per 100 caries-free permanent teeth present in the mouth at the first examination in 1943. In contrast, boys of the same age in the control area developed about 44 new DMF teeth per 100 caries-free permanent teeth. The corresponding values for girls 8 years old in 1943 were: 15

TABLE 1.—*Number of permanent teeth free of dental caries experience among 316 children examined in 1943 in 2 relocation centers, by age and sex*

Sex	Water[1]	Age in years, summer 1943							
		8	9	10	11	12	13	14	All ages
		Number of children							
Boys	Fluoride group	14	17	21	12	12	12	7	95
	Control group	4	10	4	10	8	6	10	52
Girls	Fluoride group	17	15	14	9	20	13	13	101
	Control group	7	9	10	7	13	13	9	68
		Number of caries-free permanent teeth, 1943							
Boys	Fluoride group	49	134	217	160	202	243	153	1,158
	Control group	16	69	32	126	131	115	222	711
Girls	Fluoride group	88	123	155	137	342	274	323	1,442
	Control group	25	81	118	87	226	280	204	1,021
		Number of caries-free permanent teeth per child, 1943							
Boys	Fluoride group	3.5	7.9	10.3	13.3	16.8	20.3	21.9	[2] 13.4
	Control group	4.0	6.9	8.0	12.6	16.4	19.2	22.2	[2] 12.8
Girls	Fluoride group	5.2	8.2	11.1	15.2	17.1	21.1	24.9	[2] 14.7
	Control group	3.6	9.0	11.8	12.4	17.4	21.5	22.7	[2] 14.1

[1] The water at the Arizona center contained 3 p.p.m. of fluorine; that at the California center was fluorine free.

[2] Arithmetic average of 7 age-specific rates (8–14 years).

Table 2.—*Number of permanent teeth free of dental caries experience in 1943 that showed evidence of caries experience in 1945 among 316 children in 2 relocation centers, by age and sex*

Sex	Water[1]	Age in years, summer 1943								
		8	9	10	8-10	11	12	13	14	All ages
		Number of caries-free permanent teeth, 1943								
Boys	Fluoride group	49	134	217	400	160	202	243	153	1,158
	Control group	16	69	32	117	126	131	115	222	711
Girls	Fluoride group	88	123	155	366	137	342	274	323	1,442
	Control group	25	81	118	224	87	226	280	204	1,021
		Number of teeth unaffected 1943 but affected 1945								
Boys	Fluoride group	11	18	25	54	14	20	18	31	137
	Control group	7	23	12	42	28	8	13	28	119
Girls	Fluoride group	13	20	13	46	20	42	25	35	168
	Control group	9	23	32	64	12	30	49	43	198
		Number of teeth affected 1945 per 100 teeth unaffected 1943								
Boys	Fluoride group	22.4	13.4	11.5	[2] 15.8	8.8	9.9	7.4	20.3	[3] 13.4
	Control group	43.8	33.3	37.5	[2] 38.2	22.2	6.1	11.3	12.6	[3] 23.8
Girls	Fluoride group	14.8	16.3	8.4	[2] 13.2	14.6	12.3	9.1	10.8	[3] 12.3
	Control group	36.0	28.4	27.1	[2] 30.5	13.8	13.3	17.5	21.1	[3] 22.5

[1] The water at the Arizona center contained 3 p.p.m. of fluorine; that at the California center was fluorine-free.

[2] Arithmetic average of 3 age-specific rates (8–10 years).

[3] Arithmetic average of 7 age-specific rates (8–14 years).

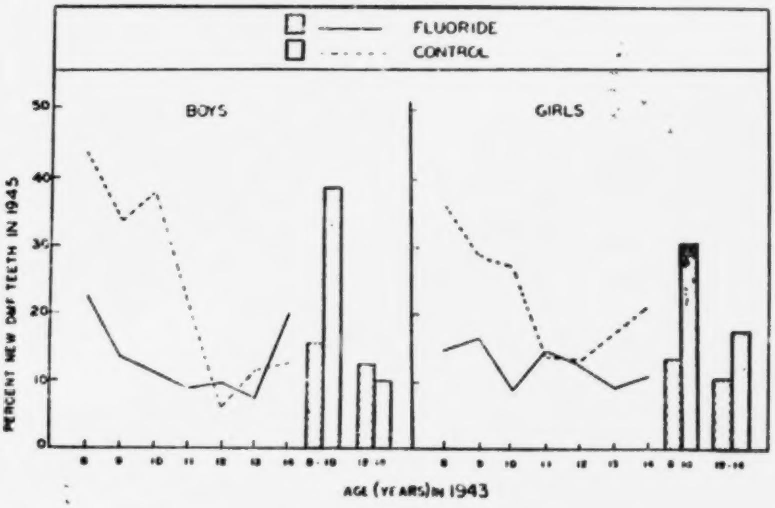

Figure 2

DMF for the fluoride group, and 36 DMF for the control group.

As shown in figure 2, the absolute differences in incidence of new caries between the fluoride and control groups tend to diminish with advancing age for both the girls and the boys. The differences became small and variable beginning at about 12 years of age in boys and at about 11 years of age in girls. In the fluoride area new caries was inhibited to a greater extent in the younger children—those 8, 9, and 10 years of age when first exposed to fluoride. In the fluoride group, boys 8 to 10 years of age at the time of the first examination developed in the subsequent 2-year period an average of about 16 new DMF teeth per 100 caries-free teeth present in 1943. Boys of similar age in the control group developed over twice as many new DMF teeth (38.2). Girls 8 to 10 years of age showed nearly the same results, averaging in the fluoride group about 13 new DMF teeth per 100 previously unaffected as compared with about 30 such teeth in the control group. Caries incidence in children who were over 11 years of age in 1943 was not affected significantly by either the presence or absence of fluorine in drinking water.

These findings lead to the conclusion that, among young children (ages 8 to 10 years) transferred to an area where the drinking water contained 3 p.p.m. of fluoride, the incidence of new caries experience in previously noncarious erupted teeth was reduced approximately 60 percent below that which would be expected on the basis of the incidence observed in the control group. The data are sufficient to indicate that exposure of the erupted permanent teeth of younger children to fluoride waters provides a larger measure of protection against caries than does the same exposure of the erupted teeth of older children. It follows therefore that, among teeth present in the mouth at the beginning of exposure to fluorine, those most recently erupted were those most protected against caries attack. Since the present report was prepared, a communication by R. Weaver [see Brit. Dent. J., *47*: 185 (1944)] has become available. This worker has arrived at similar conclusions from prevalence observations on 800 English children who had immigrated into an area where the drinking water contained 1.4 p.p.m. of fluorine. The significance of these findings with regard to the individual (different) types of teeth will be discussed in the next paper in this series of reports.

The findings reported here are not intended to constitute an endorsement for addition of as much as 3 p.p.m. of fluorine to community water supplies for the purpose of reducing caries incidence. However, the data provide information showing that addition of small amounts of fluorine to community water supplies deficient in this element effects a reduction in caries incidence in the erupted permanent teeth of residents of school age; and that such caries inhibition is most noticeable in the erupted teeth of the younger children.

DENTAL CARIES (DMF) EXPERIENCE IN RELOCATED CHILDREN EXPOSED TO WATER CONTAINING FLUORINE

By HENRY KLEIN

PURPOSE OF STUDY

The following study compares the amount of new caries developing in permanent teeth present in the mouth at the beginning of exposure to water containing fluorine with the amount of new caries developing in permanent teeth erupting during the period of exposure. It is based on seriatim dental examinations of 316 children of Japanese ancestry transferred late in 1942 from their homes in the city of Los Angeles and environs to War Relocation Centers in California and Arizona. The examinations were made with the aid of dental mirrors and explorers in the early summer of 1943, approximately six months after the arrival of the children at the Centers, and again two years later, in the early summer of 1945.

The 196 boys and girls residing at the California Center from 1943 to 1945 consumed water containing only 0.1 part per million of fluorine (a value within the error of determination). In contrast, the 120 children residing at the Arizona Center over the same time interval consumed water containing fluorine to the extent of 3 parts per million.

In a preceding report, it was shown that new caries attack in the two-year interval from 1943 to 1945 among children who were 8-10 years old in 1943 was approximately 60 per cent lower in the fluoride area than in the control area. Children of those ages were protected more in the subsequent two-year interval than were children who were 12-14 years of age at the beginning of exposure. From this finding, the conclusion was derived that, among all caries-free teeth present in the mouth when exposure to fluorine is begun, the more recent the eruption the greater is the protection against caries.

It is the purpose of the present report to examine the effect of the presence of trace amounts of fluorine in drinking water on the incidence of new caries in those individual teeth which have the highest caries susceptibility, namely, the first and second molars and the second bicuspids, and to differentiate between the caries-inhibiting effect of such waters on teeth (a) when already present in the mouth at the beginning of exposure and (b) not present in the mouth at the beginning but erupting into the mouth during that exposure.

FINDINGS

Permanent First Molars.—At the first examination in May 1943, the two groups of children—those residing in the fluoride area and those living in the control area—differed only slightly with regard to caries experience. The children in the fluoride area averaged 2.5 caries-free first molars; those in the control area, 2.8 (Table 1 and Fig. 1).

Among children 8-10 years old in 1943 who had resided in the fluoride area for the next two years, 36 per cent of the first molars caries-free in 1943 had been attacked by 1945, as compared with 58 per cent in the area with fluoride-free water (Table 2 and Fig. 2). Among permanent first molars caries-free in 1943, the percentage difference in caries incidence during the next two years in the group exposed to fluorine, as contrasted with the group using fluoride-free water, is greatest for children of the younger ages. Hence, among the children of both sexes who were 8-10 years old in 1943, those in the fluoride area developed only two-thirds as much new molar caries from 1943 to 1945 as did the control group, but, for children who were 12-14 years of age in 1943, no significant difference in caries incidence occurred for this tooth.

These findings indicate that first molars already present in the mouth of young children when fluorine exposure is begun obtain considerable protection

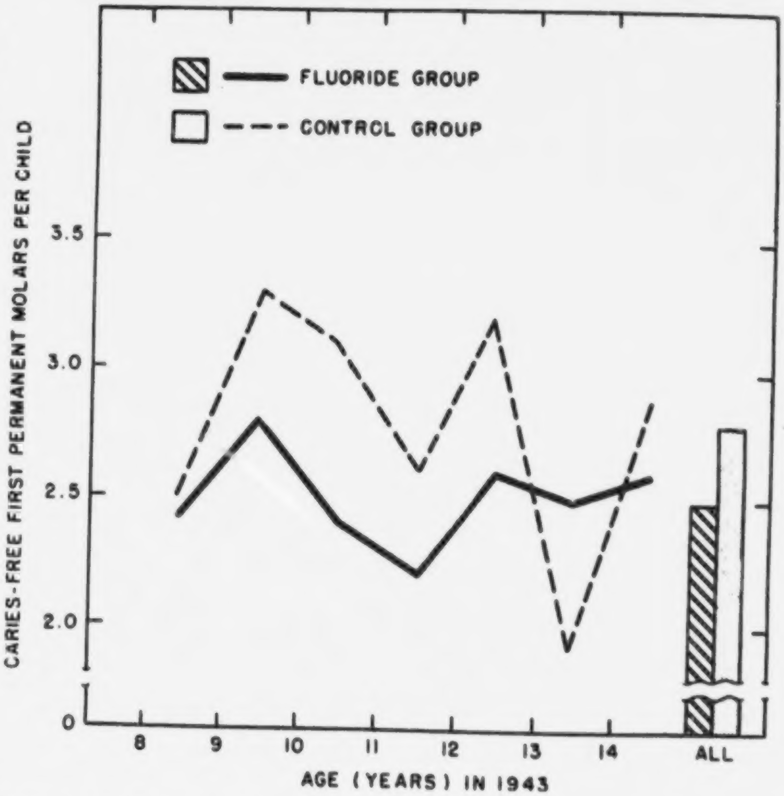

FIGURE 1.—*In the summer of 1943: Relation between age and number of caries-free permanent first molars per one hundred children of both sexes in the fluoride and control areas.*

TABLE 1.—*Erupted Permanent First Molars, 1943; Average Number of Caries-Free First Molars Among 316 Children Arranged by Age, Sex and Type of Water Consumed*

Sex	Water*	Age in 1943 (Years)							
		8	9	10	11	12	13	14	All
		Number of Children							
Boys	Fluoride group	14	17	21	12	12	12	7	95
	Control group	4	10	4	10	8	6	10	52
Girls	Fluoride group	17	15	14	9	20	13	13	101
	Control Group	7	9	10	7	13	13	9	68
Both	Fluoride group	31	32	35	21	32	25	20	196
	Control group	11	19	14	17	21	19	19	120
		Number of Caries-Free First Molars Per Child							
Boys	Fluoride group	2.1	2.6	2.6	3.1	2.7	2.7	2.6	2.6†
	Control group	3.0	3.3	3.5	2.8	3.1	2.0	3.4	3.0†
Girls	Fluoride group	2.7	3.0	2.2	1.2	2.5	2.3	2.7	2.4†
	Control group	2.0	3.3	2.6	2.3	3.2	1.8	2.4	2.5†
Both‡	Fluoride group	2.4	2.8	2.4	2.2	2.6	2.5	2.6	2.5
	Control group	2.5	3.3	3.1	2.6	3.2	1.9	2.9	2.8

* The water at the Arizona Center contained 3 p.p.m. fluorine; that at the California Center was fluorine-free (based on single samples).

† Simple arithmetic average of seven age-specific rates.

‡ Simple arithmetic average of the sex-specific rates for each group.

TABLE 2.—*Erupted Permanent First Molars, 1943-1945; Number Caries-Free in 1943 and Number and Per Cent Showing Evidence of Caries Attack (DMF) by 1945, Among 316 Children Arranged by Age, Sex and Type of Water Consumed*

Sex	Water*	Age in 1943 (Years)								
		8	9	10	8–10	11	12	13	14	12–14
		Number of Caries-Free First Molars in 1943								
Boys	Fluoride group	30	45	55	130	37	32	32	18	82
	Control group	12	33	14	59	28	25	12	34	71
Girls	Fluoride group	46	45	31	122	11	50	30	35	115
	Control group	14	30	26	70	16	41	24	22	87
Both	Fluoride group	76	90	86	252	48	82	62	53	197
	Control group	26	63	40	129	44	66	36	56	158
		Number of new DMF First Molars by 1945								
Boys	Fluoride group	11	14	24	49	13	13	6	9	28
	Control group	7	21	6	34	17	4	3	17	24
Girls	Fluoride group	13	18	11	42	3	19	6	15	40
	Control group	9	20	14	43	8	19	12	9	40
Both	Fluoride group	24	32	35	91	16	32	12	24	68
	Control group	16	41	20	77	25	23	15	26	64
		Per Cent of New DMF First Molars by 1945 Among First Molars Caries-Free in 1943								
Boys	Fluoride group	36.7	31.1	43.6	37.1†	35.1	40.6	18.8	50.0	36.5†
	Control group	58.3	63.6	42.9	54.9†	60.7	16.0	25.0	50.0	30.3†
Girls	Fluoride group	28.3	40.0	35.5	34.6†	27.3	38.0	20.0	42.9	33.6†
	Control group	64.3	66.7	53.8	61.6†	50.0	46.3	50.0	40.9	45.7†
Both‡	Fluoride group	32.5	35.6	39.6	35.9	31.2	39.3	19.4	46.5	35.1
	Control group	61.3	65.2	48.4	58.3	55.4	31.2	37.5	45.5	38.1

* The water at the Arizona Center contained 3 p.p.m. fluorine; that at the California Center was fluorine-free (based on single samples).

† Simple arithmetic average of appropriate three age-specific percentages.

‡ Simple arithmetic average of the sex-specific percentages for each age group.

against new caries. The recently erupted molars receive more protection than do first molars which have been in the mouth for longer periods.

Permanent Second Molars.—Table 3 shows the number of caries-free second molars present in the mouth at first examination and the number and percentage which showed evidence of caries attack two years later. For this tooth, data on children 12, 13 and 14 years old are available. The children of these ages of both sexes in the fluoride area had only 16 per cent of their previously caries-free second molars attacked by the summer of 1945, while those in the control area had 47 per cent attacked—a significant inhibition in the fluoride area.

Information on the number of second molars erupting into the mouth of children 12-14 years old in the two areas during the two-year exposure and the number and percentage which had been attacked by caries by the end of the exposure interval is also given in Table 3. Among children of both sexes in the fluoride area, about 4 per cent of the second molars erupting in the exposure interval were attacked by caries, whereas 26 per cent of such teeth showed evidence of caries attack by 1945 among children in the control area. From these data it may be concluded that second molars exposed to fluorine during the latter part of their calcification in the jaw before eruption or during the process of eruption into the mouth are protected more against caries attack than are second molars exposed to fluorine after eruption.

Permanent Second Bicuspids.—Table 4 provides data for children 12, 13 and 14 years old on the percentage of caries-free second bicuspids in the mouth in 1943 which were attacked by caries in the subsequent two-year interval. The incidence of new caries in second bicuspids among children of both sexes exposed to fluorine is approximately 7 per cent, as contrasted with 10 per cent for the control area, a difference which is of border-line significance.

Information on the number of second biscuspids erupting into the mouth during the two-year interval in both groups of children and the percentage of these attacked by caries is given in Table 4. Obviously, among children of the fluorine area, these teeth were exposed to fluorine before or during the process of eruption. The findings for this tooth suggest that such exposure may provide a larger measure of protection against caries attack than is provided by exposure some time after eruption has been completed.

DISCUSSION

The fluorine hypothesis in dental caries research has received considerable attention recently. Stated briefly (*3*), it proposes that an inverse relation exists between dental caries susceptibility and the ingestion of trace amounts of fluorine. It has been suggested (*4, 5*) that fluorides be added to community water supplies—and such fluorination is now being done in at least two fairly large cities—with the aim of determining whether additions of fluoride to the drinking water consumed by a population not previously so exposed will inhibit new caries attack without toxic effects.

Arnold (*6*) has reported that fluorides exert their effect on reducing caries incidence only when taken in the diet while the teeth are developing in the jaw, and that little beneficial influence in that direction can be expected in persons who are more than 5 or 6 years of age at the time that fluorine ingestion is begun. On the other hand, other experience, particularly with topical application of fluorine solutions, suggests that teeth already erupted in the mouth may be protected against caries attack even though they are not exposed to fluorine during preeruptive development (*7-9*). The findings of Weaver (*10*) are in agreement with this latter view, although he concludes that "the evidence obtained is on the whole in favor of the view that the influence of F is exerted on the teeth during the preeruptive period."

The observations recorded in the present report further support the fluorine hypothesis. The findings provide what may be considered a "pilot study" of some of the results in caries inhibition to be expected from fluorination of waters in communities previously not so exposed. Of particular interest is the finding that first and second molars and second bicuspids already erupted in the mouth are protected significantly against caries attack provided they become exposed to the fluorine-bearing waters within a short time after eruption.

The data on erupting second molars and second bicuspids, although few in number, suggest that teeth which go through the process of eruption during exposure to fluoride waters receive most protection against caries attack. This observation may be interpreted to indicate that the protective effect of fluorine is most marked if the tooth is exposed to this chemical either before eruption or during the time interval covered by the process of eruption of the tooth into the mouth.

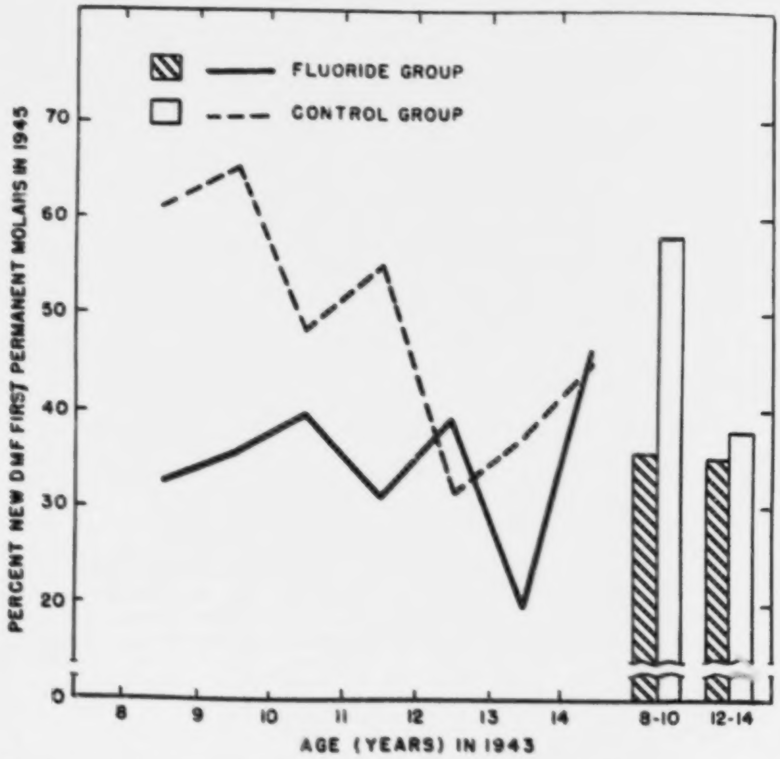

FIGURE 2.—*In the summer of 1945: Relationship between age in 1943 and percentage of new DMF permanent first molars among first molars caries-free in 1943 for children of both sexes in the fluoride and control areas.*

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Table 3.—*Permanent Second Molars, Erupted or Unerupted in 1943: Number and Per Cent Attacked by Caries (DMF) by 1945, Among 136 Children 12–14 Years of Age Arranged by Sex and Type of Water Consumed*

Sex	Water*	Erupted Second Molars in 1943				Unerupted Second Molars in 1943			
		Age (Years)				Age (Years)			
		12	13	14	All	12	13	14	All
Boys	Fluoride group	3	23	15	41	22	22	12	56
	Control group	4	12	12	28	10	15	17	42
Girls	Fluoride group	8	4	44	56	41	36	8	85
	Control group	3	15	22	40	27	30	8	65
		Number of New DMF Second Molars by 1945				Number of New DMF Second Molars by 1945			
Boys	Fluoride group	0	4	3	7	0	0	1	1
	Control group	3	4	3	10	1	3	6	10
Girls	Fluoride group	1	1	9	11	4	1	0	5
	Control group	1	8	14	23	5	14	2	21
		Per Cent of New DMF Second Molars by 1945 Among Second Molars Caries-Free in 1943				Per Cent of New DMF Second Molars by 1945 Among Second Molars Unerupted in 1943			
Boys	Fluoride group	0.0	17.4	20.0	10.5†	0.0	0.0	8.3	2.8†
	Control group	75.0	33.3	25.0	44.4†	10.0	20.0	35.3	21.8†
Girls	Fluoride group	12.5	25.0	20.5	19.3†	9.8	2.8	0.0	4.2†
	Control group	33.3	53.3	63.6	50.1†	18.5	46.7	25.0	30.1†
Both‡	Fluoride group	6.3	21.2	20.3	15.9	4.9	1.4	4.2	3.5
	Control group	54.2	43.3	44.3	47.3	14.3	33.3	30.2	26.0

* The water at the Arizona Center contained 3 p.p.m. fluorine; that at the California Center was fluorine-free (based on single samples).

† Simple arithmetic average of appropriate three age-specific percentages.

‡ Simple arithmetic average of the sex-specific percentages for each age group.

Table 4.—*Permanent Second Bicuspids, Erupted or Unerupted in 1943: Number and Per Cent Attacked by Caries (DMF) by 1945, Among 136 Children 12–14 Years of Age Arranged By Sex and Type of Water Consumed*

Sex	Water*	Age (Years)				Age (Years)			
		12	13	14	All	12	13	14	All
		Number of Second Bicuspids Caries-Free in 1943				Number of Second Bicuspids Unerupted in 1943			
Boys	Fluoride group	21	27	14	62	26	18	14	48
	Control group	9	15	31	55	23	8	7	38
Girls	Fluoride group	26	37	40	103	53	13	8	74
	Control group	16	39	27	82	33	13	1	47
		Number of New DMF Second Bicuspids by 1945				Number of New DMF Second Bicuspids by 1945			
Boys	Fluoride group	2	2	1	5	1	0	0	1
	Control group	0	1	3	4	3	0	0	3
Girls	Fluoride group	2	0	3	5	0	0	0	0
	Control group	1	9	3	13	2	1	0	3
		Per Cent of New DMF Second Bicuspids by 1945 Among Second Bicuspids Caries-Free in 1943				Per Cent of New DMF Second Bicuspids by 1945 Among Second Bicuspids Unerupted in 1943			
Boys	Fluoride group	9.6	7.4	7.1	8.0†	3.8	0.0	0.0	1.3†
	Control group	0.0	6.7	9.7	5.5†	13.0	0.0	0.0	4.3†
Girls	Fluoride group	7.7	0.0	7.5	5.1†	0.0	0.0	0.0	0.0†
	Control group	6.3	23.1	11.1	13.5†	6.1	7.7	0.0	4.6†
Both‡	Fluoride group	8.7	3.7	7.3	6.6	1.9	0.0	0.0	0.7
	Control group	3.2	14.9	10.4	9.5	9.6	3.9	0.0	4.5

* The water at the Arizona Center contained 3 p.p.m. fluorine; that at the California Center was fluorine-free (based on single samples).

† Simple arithmetic average of appropriate three age-specific percentages.

‡ Simple arithmetic average of the sex-specific percentages for each age group.

THE FAMILY AND DENTAL DISEASE. V. Caries Experience Among Parents and Offspring Exposed to Drinking Water Containing Fluoride

By **HENRY KLEIN**

It is well established that children who consume waters containing trace amounts of fluoride show, on the average, lower caries experience—that is, fewer DMF (decayed, missing and filled) teeth—than do children who drink fluoride-free waters (*1*). The reports in this connection mainly have been based on children 12–14 years of age and only preliminary information is available on the variation which undoubtedly exists (with regard to numbers of DMF teeth) among the children so exposed. Fluorination of community water supplies is being widely discussed now as a method of reducing the incidence of caries (*2*). Are some children protected more than others, and if so what factors account for such differences?

The present investigation is the fifth in a series of studies of the familial factors in dental disease and is concerned with the following specific question: Do children who drink fluoride waters all their lives (and averaging low DMF rates) show a variation in caries susceptibility related to that shown by their parents? In other terms, among children exposed to fluoride waters, do those who show the highest DMF rates have parents who have the highest caries susceptibility and, in a parallel sense, do the children who show the lowest rates have parents with the lowest susceptibility?

In a preceding publication caries experience in children was shown to be closely related to their parents' susceptibility. The now well-confirmed finding that the drinking of water containing small amounts of fluoride is associated with reduced caries attack rates leads logically to the question as to whether an environmental factor, such as fluoride in drinking water, can overcome, and perhaps obscure, the more basic factor of familial susceptibility in dental disease.

The characteristics of the population on which the present investigation is based are uniquely appropriate for the investigation. The water supply was accidentally "fluorinated" in 1927, when the water sources were changed from shallow wells (presumably fluorine-free) to deep wells containing naturally from 1.4 to 2.2 p.p.m. fluoride. As a consequence, the older population—the parents—consumed non-fluoride waters during most of the time their caries experience occurred. In contrast, their offspring—the newer generation—have been exposed to the new (fluoride) water all their lives. Hence the opportunity to examine the effects of 19 years of exposure to fluoride-water in an area where such water was not present before. Information obtained from this area may have important implications for other areas starting to or planning to fluorinate their water systems.

MATERIAL AND METHOD

Characteristics of the fluoride areas.—The communities in which the dental examinations were conducted are grouped within an area having a 15 mile radius around the town of Glassboro, in southwestern New Jersey. Excepting Woodstown, in Salem County, all the communities are located in Gloucester County a roughly rectangular area some 10 miles wide and 30 miles long, extending from the Delaware River on the west to about the middle of the State to the east, where it adjoins Atlantic County. To the north it is bounded by Camden County and on the south largely by Salem County. The County of Gloucester, except for the industrial northwestern portion, is mainly agricultural, having a very productive soil for raising fruit and vegetables.

The town of Glassboro has approximately 5,000 persons. Pitman, some 3 miles northwest, includes about 5,500 persons. Woodstown, in Salem County, about 15 miles southwest of Glassboro, includes a little more than 2,000 persons. The three communities have individual water supplies, each derived from deep wells. The well at Glassboro is 654 feet deep, at Pitman is some 500 feet, and at Woodstown is 711 feet deep. Data obtained from single samples indicate that the water in Glassboro contains 1.3 p.p.m. of fluoride, that at Pitman (2 wells) 1.4 and 1.7 p.p.m., respectively, and that at Woodstown, 2.2 p.p.m.

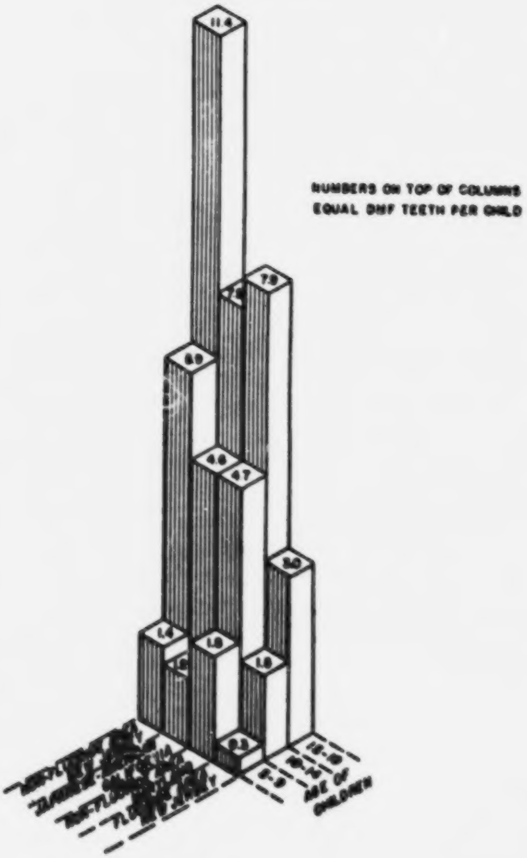
All persons who have lived in the three towns for the past 19 years have been consuming the fluoride waters. Accordingly, all children born in the area and not older than 19 years, who were permanent residents during the interval, have been exposed to the waters all their lives. On the other hand, the parents 30 years or older have been exposed to these waters since they were about 10 years old or older. This situation provides the rather unique opportunity to study the relation between the condition of teeth of parents not exposed to such waters during childhood and the condition of the teeth of their offspring exposed to the fluoride waters since birth.

Method of analysis.—The 123 fathers and 131 mothers examined were separated into three broad dental disease susceptibility groups. Evening clinics were held for examination of parents and adults. An informational letter was sent home with each school child requesting the parents to indicate the hour and evening they desired to have an examination. Although a good number of parents were examined, the strict criteria set up in the present analysis (parents of children born and brought up in the fluoride areas) account for the small number included in the present study. Among the fathers, those considered of the lowest susceptibility showed from none to as many as 14 DMF teeth, those considered of midrange susceptibility showed from 15 to

TABLE 1.—*Age distributions and DMF susceptibility groupings of 123 fathers and 131 mothers of children born and brought up in a fluoride area of New Jersey*

Parents	DMF susceptibility groups	Age of parents (years)					
		Under 30	30 39	40 49	50 and over	Unknown	All
Fathers	Low	1	25	22	1		49
	Middle	1	19	14			34
	High	1	9	13	6	11	40
	All	3	53	49	7	11	123
Mothers	Low	3	29	7		1	40
	Middle	1	6	24	26		57
	High	1	12	8	3	11	34
	All	5	47	39	28	12	131

FIGURE 1.—*Relationship between number of DMF teeth per child and age by specified geographic areas.*



19, while those considered to be highest in susceptibility had 20 to 32. Among the mothers, the low susceptibility group included all who had none to no more than 12 DMF teeth, midrange susceptibility included those having 13 to 19, while the high group included mothers having 20 to 32 DMF teeth. These groupings reflect the attempt to separate mothers and fathers into three DMF susceptibility groups of approximately equal numbers of persons. However, because the distribution of persons with various numbers of DMF teeth is not even, equalization of the groupings was not possible. The categories provide, however, a definite separation of adults (parents) into high and low susceptibility groupings with a reasonable number of parents in a middle DMF group. It will be noted that the following analysis is based only on the high and low groups.

The second, and main, step is concerned with study of the DMF rates in the children of the high and low DMF parent-susceptibility groups. The age distributions and the DMF susceptibility of the mothers and fathers are given in table 1.

FINDINGS

Children continuously exposed to the fluoride waters average, at each age, a remarkably low DMF rate. Those 5–9 years of age average 0.3 DMF tooth, those 10–14 years of age average 1.5, and the 15–19-year-old children average only 3.0. In contrast, the corresponding DMF rates for New Jersey children residing in a nonfluoride area, for Maryland children, and for Japanese-Americans of Southern California are considerably higher. (See fig. 1.)

Among the New Jersey children residing in the fluoride area and averaging a low DMF rate, a large proportion (more than 50 percent) are completely free of any evidence of caries attack. However, some of the children do have dental caries or evidence of having been attacked (in some instances to a rather marked degree). Obviously, all children exposed to fluoride waters are not protected against caries to the same extent. Is that variation in any way related to the DMF susceptibility of their parents?

Among the children born and brought up in the fluoride areas those having high DMF fathers have higher DMF rates, at almost every age, than do the children who have low DMF fathers (table 2). The children of low DMF fathers average at 10 years of age about one-half a DMF tooth and a little more than 2 DMF teeth at 16 years of age (fig. 2). On the

TABLE 2.—*Number of children and number of DMF teeth per child of specified ages and of specified parents of particular DMF susceptibility groups. All children born and brought up in a fluoride area of New Jersey*

Parent	DMF susceptibility of parent	Children of both sexes			
		Age group	Average age [1]	Number of children	DMF teeth per child [2]
Fathers	Low	5-9	7.0	30	0.5
		10-14	10.6	23	0.8
		15-19	17.0	8	2.7
	High	5-9	7.7	27	0.3
		10-14	11.7	17	1.7
		15-19	16.8	10	4.1
Mothers	Low	5-9	7.1	32	0.2
		10-14	11.8	16	0.5
		15-19	15.3	3	0.8
	High	5-9	7.9	22	0.8
		10-14	12.8	19	2.1
		15-19	16.7	5	3.8

[1] Because numbers of DMF teeth increase with advancing age, and because of the method of selection of the parent susceptibility groups, the high DMF parents tend to be older in age and therefore the average age of their children tends to be higher than that of children of low DMF parents. Comparison of the DMF rates of the children of the two groups of parents (high and low DMF) takes account of these age differences as shown by the data plotted in fig. 2.

[2] Simple arithmetic average of the sex-specific rates.

other hand, the children of high DMF fathers average 1 DMF tooth at 10 years of age and nearly 4 DMF teeth at 16 years of age.

The children (table 2 and fig. 2) of high DMF mothers average higher DMF rates at every age than do the children who have mothers with a low DMF rate. For example, among the children whose mothers' DMF level is known, those of low DMF mothers average less than one-half a DMF tooth at 10 years of age, and less than 1 DMF tooth at 15 years of age. In contrast, the children of high DMF mothers average nearly 1½ DMF teeth at 10 years, and about 4½ DMF teeth at 15 years of age.

Assortative mating.—Analysis of findings on children of marital pairs where husband and wife are both of the same DMF susceptibility groups (like-matings) cannot be treated extensively here since the paired couples total only 95 in number. Of these, 51 couples or 53.6 percent represented marriages of like with like; that is, low DMF husbands with low DMF wives, middle with middle, and high with high. The children of these like-matings total 72, a group too small to provide stable DMF rates for single-age groups.

On the basis of random selection, marriages of like with like in this group would be expected in 33.3 instances out of every 100 pairings. Accordingly, and on the basis of these data, from the dental viewpoint, marriages of persons of like DMF levels occur 19 percent more frequently than would be expected if the mate selection were random. Similar findings have been described for a group of more than 1,000 marital pairs of Japanese-Americans.

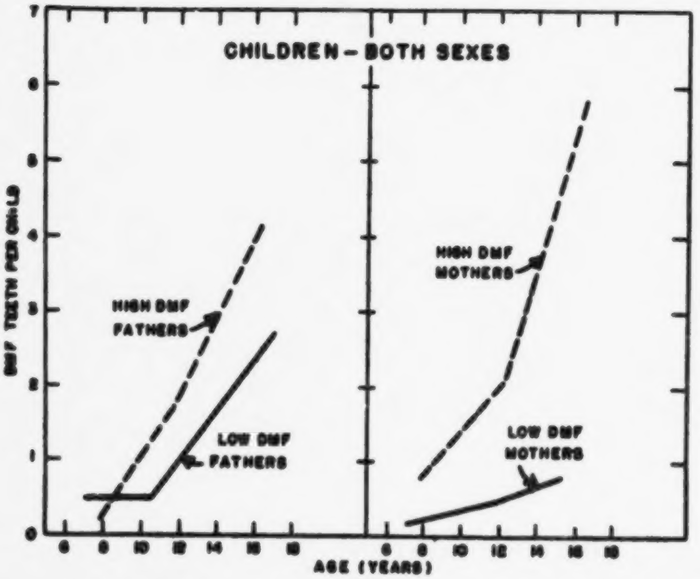


FIGURE 2.—*Relationship between number of DMF teeth per child and the dental disease susceptibility levels of parents among a population continuously exposed to drinking waters containing fluoride.*

COMMENT

Although the data presented here are fewer than desired, they are sufficient to indicate that children with continuous exposure to fluoride water since birth are as a group protected against caries attack. However, the degree of protection varies among the exposed. Those who are protected least are children whose parents show the highest tendency toward dental disease, while those who are protected most are children whose parents show the lowest tendency to be attacked by dental disease.

Expressed in other terms, it appears from these data that caries experience in children is determined, among other factors, by familial susceptibility. Exposure to an environmental factor (fluorine in the diet) will reduce the amount of caries attack, but not sufficiently to obscure the influence of the familial factor which may be constitutional in origin.

For areas where fluorination of water supplies is contemplated or now under way, the findings of the present study are peculiarly pertinent since they are based on an experience of 19 years of exposure to fluorine. The data suggest that the control of dental caries may be not alone a problem of environment (fluorine in the diet) but perhaps also a problem of constitution (quality of parent stock).

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FLUORINE IN DRINKING WATER: ITS EFFECT ON DENTAL CARIES

FRANCIS A. ARNOLD, JR.

During the last decade dentistry has been presented with evidence suggesting a practical method for partially controlling dental caries in large population groups; that is, by means of fluorination of domestic water supplies. Such a suggestion originated principally from studies of the effects of fluoride-bearing waters on the prevalence of caries. In view of the fact that hitherto no effective means of mass control of this disease has been known, both the dental profession and the laity are extremely interested in the evidence presented. Often the hopes and expectations of both groups go beyond logical interpretation of the scientific evidence.

It is the purpose of this paper to review briefly some of the major points of evidence reported to date, with special reference to the epidemiologic studies of water-borne fluorides. A more complete review of the dental caries-fluorine relationship can be found in the recent publication of the American Association for the Advancement of Science (*1*).

Prior to 1938, most dental research in regard to water-borne fluorides centered on the problem of dental fluorosis. Today the cause of this disease and the methods of its prevention are known. Of special importance in the dental caries-fluorine phenomenon is the fact that the concentrations of fluorides that produce fluorosis also are known. Aside from the establishment of these basic facts, additional knowledge has been obtained. As stated by Dean (*2*), "Studies in endemic dental fluorosis provided the proving ground whence evolved many of the ideas and methodologies later utilized in the studies designed to outline the epidemiological characteristics of the fluorine-dental caries relationships."

Before the etiologic agent of dental fluorosis, fluorine, was known and prior to the first report of low caries rates in endemic areas of dental fluorosis, mention was made of the fact that caries was no more prevalent, and probably less so, in persons who exhibited evidence of fluorosis (*3-5*). Special interest of dental research workers was focused on the caries-fluorine relationship a decade ago after fluorine was recognized to be the cause of mottled enamel and the earlier observations were supported by the results of both laboratory and epidemiologic studies.

EPIDEMIOLOGIC STUDIES

After Dean (*6*) had reported concerning the lowered dental caries rates in areas of endemic dental fluorosis in South Dakota, specific epidemiologic studies were inaugurated to gain further knowledge of this phenomenon. These studies were designed to check by detailed local investigations the relation between fluorosis and caries.

The observations were limited to specific groups according to age and sex. White school children, aged 12 to 14 years, were selected. Since it was logical to assume that this phenomenon might be related to differences in domestic water supplies, only children who used continuously one common water supply were included. The cities selected for study had domestic water supplies which met the necessary prerequisite as to fluoride content and had made no changes in their water supplies for a period greater than the age of the children studied. Factors such as dietary habits (especially carbohydrate intake), latitude and sunlight were taken into account in so far as possible. Complete details of the epidemiologic technics employed in the selection of children and areas of study have been presented by Dean (*7*).

The first of these investigations was made in four cities in north central Illinois (*8*). Two of these cities, Galesburg and Monmouth, had communal water supplies which contained 1.8 and 1.7 parts of fluoride per million, respectively. Quincy and Macomb, the control cities, used domestic water supplies which were fluoride-free; that is, they contained less than 0.2 p.p.m. of fluoride.

The prevalence of dental caries in the children of these four communities differed greatly. For example, the average 13 year old child in Galesburg had experienced caries in only about two permanent teeth, but in Quincy the average 13 year old child had about six teeth showing caries experience. It also was noted that in Galesburg and Monmouth approximately 36 per cent of the children were caries-free as compared with 14 percent in Macomb and 4 percent in Quincy.

Other interesting findings of this study were the differences in the distribution of high and low Lactobacillus acidophilus counts, the striking differences in proximal and smooth surface caries and the relation between macroscopic fluorosis and dental caries. In the case of the last, it was observed that the lowered caries rates in Galesburg children were not associated with the presence of dental fluorosis by itself. This finding confirmed the earlier observations by Dean (*6*).

The evidence from this study together with other information available at that time pointed to water supplies and presumptively to the fluoride content of those water supplies as the primary associated factor. This raised the question of whether a decreased prevalence of dental caries is associated with the use of water supplies whose fluoride content is less than the concentration necessary to produce any significant amount of dental fluorosis.

In order to answer this question a detailed study was made of school children in eight suburban Chicago communities. The communities and the children were selected in the manner previously described (*9*). The results of this study confirmed the findings at Galesburg and Quincy in regard to the marked differences in the prevalence of dental caries between children using fluoride waters as compared with children using fluoride-free water.

As shown in table 1, the caries experience rates in Elmhurst, Maywood, Aurora and Joliet, whose public water supplies contain 1.8, 1.2, 1.2 and 1.3 p.p.m. of fluoride, respectively, were 252, 258, 281 and 323, respectively. The children of Evanston, Oak Park and Waukegan, using fluoride-free water, showed caries experience rates of 673, 722 and 810, respectively. The results of the examinations at Elgin, where the public water supply contains about 0.5 p.p.m. of fluoride, indicate a caries experience rate (444) that is about midway between that of the fluoride and fluoride-free areas.

The observations in this study were suggestive of an inverse variation of dental caries and the fluoride concentration of the domestic water. It also was noted that the inhibitory factor, presumably fluorine, was operative at concentrations below what is now the accepted permissible standard for water, 1.5 p.p.m. (*10*). At these low concentrations dental fluorosis is no problem. Evidence of this fact was obtained in Aurora where although 15 per cent of the children were classed as having fluorosis, only 5 percent of all the teeth and only about 0.4 percent of the anterior teeth showed even so much as the mildest forms of fluorosis. This minor amount of affection, which is noticeable only to a trained observer, is of no esthetic or public health significance.

As may be noted in table 1, there were differences other than the fluoride content in the water supplies of the communities studied. For example, the cities in which the caries rates were low used well waters while those with high caries prevalence rates used surface (Lake Michigan) water. Thus, comparisons were made on water supplies of varying mineral content. In order to check this variable and to confirm the observation regarding the effect of the lower ranges of fluoride concentrations (1 p.p.m. and less), epidemiologic studies in other communities were conducted. By the use of similar technics of examination and the same criteria of selection, children 12 to 14 years of age in nineteen additional communities were studied.

The communities selected had water supplies which varied from a high fluoride content with a low mineral content to fluoride-free waters with a high mineral content. The sources of these water supplies were deep wells, lakes and rivers, and cisterns. Including the children in the eight Chicago suburbs, a total of 8,576 children in 27 cities of 8 states have been studied. The results of these examinations are shown in the illustration and in table 2. More complete details concerning these study areas were presented by Dean, Arnold and Elvove (*11*).

The data suggest the following conclusions: First, children who are born and reared in communities whose domestic water supplies contain increased amounts of fluorine (more than 0.5 p.p.m.) ex-

TABLE 1.—*Summary of dental caries findings in 2,832 selected white children, aged 12 to 14 years, in eight suburban Chicago communities in relation to the fluoride content of the public water supply (9)*

	City or village		Elmhurst	Maywood	Aurora	Joliet	Elgin	Evanston	Oak Park	Waukegan
Sampling	Total number of 12-14 year old children present at time of sampling		633	873	1,625	1,412	1,030	2,125	1,662	1,354
	Number of 12-14 year old white children whose histories on repeated questioning indicated continuity of exposure and who were examined		170	171	633	447	403	256	329	423
	Percentage of the total present who were examined		26.9	19.6	39.0	31.7	39.1	12.0	19.8	31.2
	Source		Deep wells					Lake Michigan		
Water supply	Permanent hardness in parts per million		323.4	75.0	328.5	349.3	102.6-	131.0	132.2	134.4
	Mean fluoride (F) content, 1939-1940, in parts per million		1.8	1.2*	1.2	1.3	0.5	0.0	0.0	0.0
Clinical examination	Dental caries experience, permanent teeth, per 100 children examined		252	258	281	323	444	673	722	810
	Dental caries experience, proximal surfaces, superior incisors, per 100 surfaces		0.60	0.59	0.78	1.3	4.1	10.7	9.0	17.7
	First permanent molar mortality, per 100 children examined		11.8	11.7	14.5	19.5	20.3	42.6	31.0	79.9
	Percentage of children with no dental caries experience		25.3	29.8	23.5	18.3	11.4	3.9	4.3	3.1
	Percentage incidence of endemic dental fluorosis (mottled enamel)		40.0	33.3	15.0	25.3	4.2	1.6	0.6	0.2
Bacteriology	Percentage of those examined bacteriologically whose salivary L. acidophilus counts were:	Negative and <100	35.7	39.6	38.2	35.6	21.2	15.4	18.2	14.9
		30,000 and >	24.7	20.9	25.6	26.6	33.2	41.8	43.8	54.6

*There is both presumptive and direct evidence that prior to a few years ago the Maywood water contained probably 1.4-1.6 p.p.m. of fluoride (F).

perience less dental caries than do children living in areas where the domestic water supply is fluoride-free (tables 1 and 2). Second, this phenomenon is operative whether or not the teeth show macroscopic evidence of fluorosis. Third, the decreased prevalence of dental caries is evident in areas where the fluoride content of the water is below those levels which produce dental fluorosis of any esthetic significance.

A careful analysis of the data strongly suggests fluorine as the active inhibitory factor present in these water supplies.

As stated previously, factors other than those present in the water supplies which probably could have influenced the dental caries experience were taken into account and in so far as possible were equalized by careful selection of the study areas. As for the variability in water supplies, one characteristic other than fluoride which has been suggested as lowering dental caries experience is the total hardness of the water. In the study of the 27 cities, however, there is little evidence of correlation between the total hardness of the water and caries prevalence (table 2). For example, Middletown and Zanesville, Ohio, and Elkhart, Ind., have waters of relatively high total hardness (329, 291 and 220 p.p.m., respectively) and show caries experience rates of 703, 733 and 823, respectively. On the other hand, Colorado Springs, Colo. and Maywood, Ill., using waters of much lower total hardness (27 and 75 p.p.m., respectively) had lower caries rates (246 and 258, respectively). The one variable, however, which does correlate with caries prevalence is the fluoride content of these water supplies.

Attention is called to the possibility that other factors or combinations of factors associated with water may play a role in the carious process or in the caries-fluorine relationship. At present comparatively little information is available regarding the physiologic role of such a major part of our daily diet as our drinking water. It is known that there is a great variation at least in the chemical composition of the different waters; further study may disclose physiologic effects resulting from some of these variables.

In addition to indicating that fluorine is the active inhibitory factor, the observations in the twenty-seven cities also suggest that almost full benefit is obtained if the fluoride content is only about 1 p.p.m. At this low range of fluoride concentration the dental fluorosis index is less than 0.5 (see illustration). A description of the method (*12*) of establishing a fluorosis index has been given by Dean. The problem of dental fluorosis in these areas is negligible as indicated by the observation in Aurora, Ill., as previously described. Increasing the fluoride content much above 1 p.p.m. produces little additional inhibition of caries but does materially increase the amount and degree of fluorosis (*13*).

In studies in this and other countries observations of a similar character have been made. Weaver (*14*) made a study of dental conditions in the British Isles and found that the children of South Shields had only about half as much dental caries experience as did the children of North Shields, which is just across the River Tyne. He attributed the variability in caries prevalence to differences in the domestic water supply, specifically to the fluoride content. The water supply of South Shields contained 1.4 p.p.m. of fluoride and that of North Shields contained 0.25 p.p.m. Ockerse (*15*) reported similar findings in South Africa.

This evidence, like most epidemiologic evidence, is presumptive in character (*16*), but in addition to these studies on human beings there are reports from laboratory research which indicate that fluorine influences the carious process in experimental animals. A number of authors have shown that induced caries in the molars of rats may be inhibited by the addition of fluorides to the drinking water or the food and that this caries-inhibiting action is related to the amount of fluoride ingested. Also, dental caries in Syrian hamsters has been influenced in a like manner. A review of experimental caries and fluorides was made by Hodge and Sognnaes (*17*).

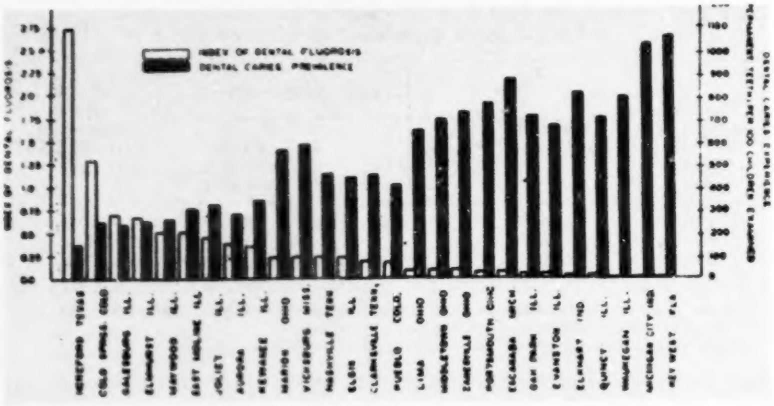
Although it is not known that dental caries in human beings is comparable to induced caries in animals, such laboratory evidence lends weight to the presumption that fluorine is the active ingredient in the water supplies in areas of decreased caries rates.

MECHANISM STUDIES

At the present time the mechanism of the action of fluoride waters on the carious process is not known; however, the limited information available sheds some light on this phenomenon. One of the more important observations made in connection with the epidemiologic studies is in regard to changes in the oral bacteriologic flora. It is a generally accepted belief that dental caries activity can be diagnosed by determining the number of Lactobacilli in the saliva. The oral flora of persons in whom caries is active shows a large number of Lactobacilli per cubic centimeter, but in persons in whom caries is inactive or absent the oral flora is negative for Lactobacilli, or the organism is present only sporadically and in low numbers.

The results of such determinations in the fluoride and nonfluoride areas, shown in table 3, indicate that there is a definite difference in the percentage distribution of Lactobacillus counts in the saliva of children included in some of the epidemiologic studies (*8, 9, 18*). The most striking differences were noted in the 0–100 range (inactive caries) and in those counts over the 20,000–30,000 range (active caries). The differences in the counts paralleled the differences in caries experience rates observed in these children.

Cities using waters with a fluoride content of more than 1 p.p.m. averaged 37.5 percent in the 0–100 range compared with 17.8 percent in the fluoride-free areas (table 3). In the intermediate fluoride level (0.5 p.p.m.) 21.2 percent of the counts were in the low range. Conversely, the high counts (20,000 and more) were 28.7 percent in the fluoride areas and 52.4 percent in the fluoride-free areas. These differences in the percentage distribution of high and low Lactobacillus counts suggest that the caries-fluorine relationship may be based on action of the fluoride waters on the oral flora. In other words, they point to a decrease in the activating forces of the carious process. This result would seem to indicate a theory of action opposed to that suggested by laboratory evidence on chemical composition of the teeth (*19*).



Relation between the amount of dental caries in the permanent teeth of 8,576 white children 12 to 14 years old in 27 cities of 8 states and the index of dental fluorosis.

Additional information on the mechanism of action of fluoride waters has been gained by studying persons who gave a history of change from fluoride to fluoride-free water supplies or vice versa. In Bauxite, Ark., it was observed that children whose teeth were formed on a fluoride water and erupted into a fluoride-free environment experienced less dental caries than children of a neighboring community who had used fluoride-free water throughout life (*20*). This finding is in accord with the results reported by Deatherage (*21*) on selectees in Illinois.

Observations somewhat opposite in character were made in Maywood, Ill., Garrettsville, Ohio and Escanaba, Mich. (*22, 23*). In Maywood a study was made of children who had moved to that city at varying ages from areas using fluoride-free Lake Michigan water. With the exception of those children who had moved to Maywood prior to 4 years of age, there was no significant evidence of any inhibition of caries incidence following the change from a fluoride-free to a fluoride environment. At Garrettsville, Ohio, and Escanaba, Mich., the respective water supplies were changed from fluoride-free water to that containing increased amounts of fluorine. Dental and bacteriologic examinations in Garrettsville and bacteriologic examinations in Escanaba gave no indication of a change in caries activity in children whose teeth had formed on fluoride-free water.

Analysis of these results suggests the necessity of an increased ingestion of fluoride from birth or at least during the period of tooth formation in order to obtain beneficial effects. These results, however, are not entirely in accord with the findings of Deatherage (*24*).

TABLE 2.—*Summary of dental caries and fluorosis findings in 8,576 selected white school children, 12 to 14 years of age, in twenty-seven cities of eight states in relation to fluoride content, total hardness and source of domestic water supply*

City	Children examined	Clinical			Water supply		
		Caries experience in permanent teeth		Percent children with fluorosis	Fluoride, p.p.m.	Total hardness, p.p.m.[1]	Source
		Percent with none	Number per 100 children				
Hereford, Texas	60	38.3	147	100.0	3.1	383	Deep wells
Colorado Sprgs, Colo	404	28.5	246	73.8	2.6	27	Surface (Pike's Peak)
Galesburg, Ill	273	27.8	236	47.6	1.9	247	Deep wells
Elmhurst, Ill	170	25.3	252	40.0	1.8	323	Do.
Maywood, Ill	171	29.8	258	33.3	[2]1.2	75	Do.
East Moline, Ill	152	20.4	303	31.6	[2]1.2	276	Do.
Joliet, Ill	447	18.3	323	25.3	1.3	349	Do.
Aurora, Ill	633	23.5	281	15.0	1.2	329	Do.
Kawanee, Ill	123	17.9	343	12.2	0.9	445	Do.
Vicksburg, Miss	172	8.7	587	11.0	0.2	94	Yazoo and Miss. River.
Nashville, Tenn	662	9.4	461	8.6	0.0	79	Cumberland River.
Clarksville, Tenn	60	16.7	458	8.3	0.2	89	Do.
Pueblo, Colo	614	10.6	412	6.5	0.6	302	Arkansas River.
Marion, Ohio	263	5.7	556	6.1	0.4	209	Deep wells.
Elgin, Ill	403	11.4	444	4.2	0.5	103	Do.
Lima, Ohio	454	2.2	652	2.2	0.3	223	Impounded surface.
Evanston, Ill	256	3.9	673	1.6	0.0	131	Lake Michigan.
Zanesville, Ohio	459	2.6	733	1.5	0.2	291	Deep wells.
Escanaba, Mich	270	1.1	877	1.5	0.2	123	Lake Michigan.
Portsmouth, Ohio	469	1.3	772	1.3	0.1	80	Ohio River.
Middletown, Ohio	370	1.9	703	1.1	0.2	329	Deep and shallow wells.
Oak Park, Ill	329	4.3	722	0.6	0.0	132	Lake Michigan.
Elkhart, Ind	278	1.4	823	0.4	0.1	220	Deep wells.
Quincy, Ill	330	2.4	706	0.3	0.1	88	Mississippi River.
Waukegan, Ill	423	3.1	810	0.2	0.0	134	Lake Michigan.
Michigan City, Ind	236	0.0	1,037	0.0	0.1	141	Do.
Key West, Fla	95	0.0	1,070	0.0	[3]0.1	[3]36	Cisterns.

[1] Total hardness calculated in accordance with *Standard Methods for the Examination of Water and Sewage*. New York: American Public Health Association, 1936.

[2] There is both presumptive and direct evidence that these water supplies contained about 1.5 p.p.m. of fluoride prior to a few years ago.

[3] Average of 5 water samples from cisterns considered typical of those used by most Key West inhabitants.

DISCUSSION

A logical assumption, in view of the evidence so far presented, is that dental

Table 3.—*Percentage distribution of oral Lactobacillus counts on 12 to 14 year old children living in fluoride and nonfluoride areas*[1]

Fluoride content of domestic water, p.p.m.	Children examined	L. acidophilus counts		Dental caries experience (rate per 100)[2]
		0-100, percent	20,000 and more, percent	
1.0	1,052	37.5	28.7	280
0.5	250	21.2	40.0	444
0.0	1,087	17.8	52.4	760

[1] Includes children from the following cities: Quincy, Evanston, Oak Park, Waukegan, Elgin, Elmhurst, Maywood, Aurora, Joliet and Galesburg, Ill., and Escanaba, Mich.

[2] Based on all children given dental examination in respective communities.

caries may be controlled by fluorination of water supplies. The unusually high prevalence of this disease makes it particularly suited to a type of control measure which is on a communal basis and is not dependent on individual volition.

Fluorination of water is economical. According to Harris (*25*), the per capita cost per year is about 7½ cents. This figure varies, depending on the price of the fluoride used and the amount already present in the water supply.

The results that might be expected from such a control measure were discussed in a previous article (*26*).

In comparing the dental caries picture of the children of Aurora where the domestic water contains 1.2 p.p.m. of fluoride with that of children using fluoride-free water, it was noted that in Aurora (1) about six times as many children had had no dental caries experience; (2) there was about 60 percent less dental caries experience; (3) there was a decrease of almost 75 percent in mortality of the permanent first molar, and (4) approximately 95 percent less caries occurred on the proximal surfaces of the four upper incisors.

Attention is called to the fact that the evidence to date in regard to the effect of water-borne fluoride on human dental caries is of an epidemiologic nature. At present one can only assume that the ingestion of a water which has fluoride added artificially will produce the same effects as the ingestion of a natural fluoride water. All evidence suggests that it will, but the final answer to this question awaits the results of research studies now in progress. In eight communities in the United States and Canada (Grand Rapids, Mich.; Newburgh, N.Y.; Midland, Mich.; Brantford, Ont.; Sheboygan, Wis.; Ottawa, Kans.; Evanston, Ill., and Marshall, Tex.) fluorine is being added to the domestic water supply for the purpose of studying the effect on dental caries. The first of these communities to start adding the fluorine was Grand Rapids, Mich. This study is sponsored by the U.S. Public Health Service with the cooperation of the Michigan Department of Health and the University of Michigan. Fluorination was begun in January 1945, and to date no major mechanical difficulties have been encountered. More complete technical details of this problem were given by Harris (*25*).

The influence of fluorination on the teeth of present inhabitants of these communities poses another question. Both Deatherage (*24*) and Klein (*27*) have reported beneficial effects in persons who had moved from supposedly fluoride-free areas to fluoride areas. However, the limited epidemiologic evidence in Maywood suggests that fluorination of water supplies may have little or no effect on the carious process in teeth formed on fluoride-free waters.

In view of the latter evidence, an abrupt beneficial change in dental caries incidence would not be expected to follow the fluorination of a domestic water supply. Preliminary tabulation of results of annual examinations in Grand Rapids supports this contention. It appears difficult to explain, however, why numerous topical applications of fluoride, as would occur from the ingestion of fluorinated waters, would not have over a period of years a beneficial effect somewhat similar to topical application of stronger solutions of fluoride. It may be that the diagnostic methods used were not critical enough to demonstrate the minor changes in the incidence of caries and that more time will be required for clarification of the results.

Attention is called to the fact that other factors such as climatic conditions, culinary habits and unusual mineral constituents of water supplies may play an important role. Further research studies together with the results of projects now in operation should shed light on some of these aspects of the problem.

SUMMARY

Epidemiologic evidence indicates that the ingestion during the formative period of the teeth of natural waters containing as little as 1 part per million of fluorine is accompanied by a decrease in the prevalence of dental caries. Presumptive evidence points to fluorine as the active inhibitory agent and suggests the possibility of adding controlled amounts of fluorine to water supplies as a practical method of preventing or inhibiting caries.

Studies of such a procedure are now in progress, but final evaluation must be deferred during the time required for teeth to develop and erupt under this changed environment. At present it is deemed advisable to await the results of these research projects before an unqualified recommendation for mass fluorination of water supplies is made.

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DENTAL EFFECTS OF ACCIDENTALLY FLUORINATED WATER. I. Dental Caries Experience in Deciduous and Permanent Teeth of School Age Children

By Henry Klein

It is established fairly well now that children consuming waters containing trace amounts of fluorine (1 to 2 parts per million) experience less dental decay than do comparable children taking fluoride-free waters. On the basis of such findings, several communities in the United States are deliberately introducing sodium fluoride into their public water supplies in order to study over a ten year period the effects of the artificial fluorination of public water supplies on the dental caries experience of the population (*1*). Accordingly, were a community to be discovered where water supplies had become fluorinated more than a decade ago, perhaps significant findings could be anticipated with regard to the effects of artificial fluorination.

Such an area has been identified in New Jersey where the community water supplies may be presumed to have contained from 1.2 to 2.2 p.p.m. of fluoride for nineteen years. The fluorination in this area was accomplished accidentally when the communities changed in 1927 from a fluoride-free water derived from wells approximately 100 to 130 feet in depth to fluoride-bearing waters derived from wells tapping the Raritan stratum at a depth of approximately 500 to 700 feet. This area provided an opportunity to study systematically the effects, dental and otherwise, of an exposure of nearly two decades to waters containing fluorides to the extent of 1.2 to 2.2 p.p.m.

In order to provide a complete set of data for the study, a control area located in close proximity was selected. The waters now delivered in the control area are derived from the same stratum as were the waters delivered to the fluoride communities previous to 1927. These waters are fluoride-free. However, the unusual intensity of dental caries attack in the control communities raises the question as to whether these latter waters contain principles which favor dental decay (*2*).

METHOD

The fluoride area includes three communities, Glassboro, Pitman and Woodstown. The nonfluoride area includes two communities, Clayton and Williamstown. Glassboro and Pitman, of approximately 5,000 population each and separated by a distance of about 3 miles, are located at the geographic center of Gloucester County. They now receive their water supply from fairly deep wells; the Glassboro well is approximately 600 feet deep and has been in use since 1927. The water supply at Pitman is obtained from two deep wells each about 500 feet deep. The one supplying the larger proportion of the population has been in use since 1927; the other was placed in operation in 1937. Of persons who consume water from this well, only those younger than 10 years of age were included in this study.

Before 1927 the water supplied to Glassboro was obtained from wells approximately 100 feet deep located at Clayton, whose water supply also was obtained from the same source. Previous to 1927 the water supplied to Pitman was obtained from wells under 200 feet in depth. The 1918 annual report of the New Jersey State Health Department indicates a deep well in Pitman. Although such a well existed it was used privately and not as a community water source.

Williamstown derives its water from wells about 130 feet deep. Both Clayton and Williamstown presumably now tap the same stratum and receive the same type of water as was delivered at Glassboro and Pitman previous to 1927. The water at Woodstown is obtained from a well driven in 1927 to a depth of over 700 feet. Before 1927 the water supply at Woodstown was obtained from relatively shallow wells and presumably was fluoride-free. Single sample analyses of these several waters indicate the following fluoride content in parts per million (May 1946):

Woodstown	2.2
Glassboro	1.3
Pitman—Well 1	1.7
Pitman—Well 2	1.4
Williamstown	0.1 (fluoride-free)
Clayton	0.1 (fluoride-free)

More than 3,100 school children were examined in all the communities during the winter of 1945 and spring of 1946 (*3*). As shown in table 1, 944 of these children were lifetime residents (born and reared) of the fluoride area and used the city water supply all their lives; 340 were born and reared in the nonfluoride area and used that city water all their lives; 599 other children who attended the schools in either the fluoride or nonfluoride areas were born and reared in their respective areas but had used water from shallow wells at their individual farm homes. Children totaling 882 were migrants into the fluoride area (coming from outside the nonfluoride areas) and 425 children were migrants into the nonfluoride areas (coming from outside the fluoride areas).

Table 2.—*Number of deciduous teeth, carious or filled, per 100 deciduous teeth present in children born and reared in specified areas*

Locality	Water source	Sex	Age at survey (years)							
			5	6	7	8	9	10	11	12
Non-fluoride	City	Boys	20.2	22.7	50.6	43.7	47.7	45.6	28.2	37.9
		Girls	16.3	19.7	35.2	40.5	46.3	35.0	38.5	100.0
		Both*	18.3	21.2	42.9	42.1	47.0	40.3	33.4	69.0
	Home well	Boys	—	26.5	60.3	4[illegible]6	57.9	38.8	81.3	100.0
		Girls	—	31.2	31.7	35.[illegible]	44.1	60.6	95.2	50.0
		Both*	—	28.9	46.3	39.0	51.0	49.7	88.3	75.0
Fluoride	City	Boys	3.0	5.1	12.5	13.1	22.7	15.1	22.7	26.8
		Girls	2.6	9.4	8.8	11.7	22.1	21.9	27.6	46.1
		Both*	2.8	7.3	10.7	12.4	22.4	18.5	25.2	36.5
	Home well	Boys	15.9	29.4	47.2	35.5	59.6	29.4	33.3	63.6
		Girls	10.4	18.4	35.6	47.8	43.6	36.0	52.6	—
		Both*	13.2	23.9	41.4	41.7	51.6	32.7	43.0	—

* Simple arithmetic means of sex-specific rates.

In this report, the extent to which caries attacks the children is measured in terms of three different rates. The amount of caries experienced in the deciduous teeth is measured as the number of such teeth decayed plus the number filled (numerator) divided by the number of deciduous teeth present in the mouth, times 100. This is a caries prevalence rate which is calculated for each single year of age from 5 through 12 years.

Caries experience in the permanent teeth is expressed in terms of two different rates. The first is a prevalence rate: the number of permanent teeth decayed, missing and filled (*4*) (numerator) divided by the number of children. This rate is derived for each single year of age from 6 through 18 years. The second rate used for the permanent teeth is an incidence rate: the amount of caries experienced (for particular five-year age groups of children covering the range from 5 through 19 years) per 100 person-years of age. This latter rate describes the amount of caries experienced per 100 aggregate years of age of the children in the given age group. The rate is used here because it takes account of and controls the slight differences in the average ages of the children included in a 5-year age group but differing in duration of exposure to the drinking water in the several areas. On the other hand, the caries prevalence rate (DMF teeth per child) is used, as indicated earlier, for

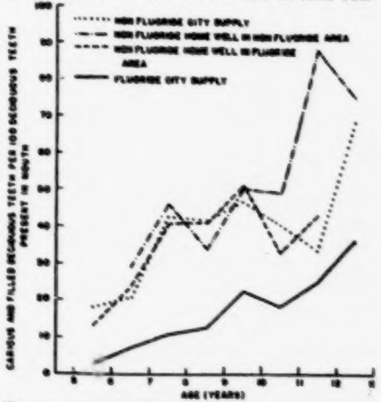

Figure 1.—*Relation between age and number of carious and filled deciduous teeth per 100 deciduous teeth present.*

Table 1.—*Children included in survey*

Locality	Residence history	Water source	Sex	Age at survey (years)														
				5	6	7	8	9	10	11	12	13	14	15	16	17	18	All
Non-fluoride	Born and reared	City	Boys	5	10	13	19	21	15	10	12	20	12	11	8	4	3	163
			Girls	5	18	17	24	20	9	11	14	12	11	16	7	11	2	177
			Both	10	28	30	43	41	24	21	26	32	23	27	15	15	5	340
		Home well	Boys	—	10	10	7	5	9	9	7	11	9	23	8	11	5	124
			Girls	—	6	13	6	8	8	5	11	9	10	22	18	7	11	134
			Both	—	16	23	13	13	17	14	18	20	19	45	26	18	16	258
	Migrants	City	Boys	5	17	12	21	18	22	14	16	15	22	27	18	17	3	227
			Girls	2	16	14	11	13	12	18	12	13	25	21	19	14	8	198
			Both	7	33	26	32	31	34	32	28	28	47	48	37	31	11	425
Fluoride	Born and reared	City	Boys	37	35	63	46	53	43	30	26	37	30	27	32	21	7	487
			Girls	40	44	43	44	41	29	27	30	25	28	38	37	22	9	457
			Both	77	79	106	90	94	72	57	56	62	58	65	69	43	16	944
		Home well	Boys	6	5	8	5	9	8	8	8	7	24	34	30	14	4	170
			Girls	9	5	8	13	9	4	7	7	16	19	24	29	17	4	171
			Both	15	10	16	18	18	12	15	15	23	43	58	59	31	8	341
	Migrants	City	Boys	15	26	34	30	39	28	42	21	34	46	46	33	33	6	433
			Girls	8	36	27	38	18	27	40	35	32	50	53	32	36	17	449
			Both	23	62	61	68	57	55	82	56	66	96	99	65	69	23	882
All	All	All	Boys	68	103	140	128	145	125	113	90	124	143	168	129	100	28	1604
			Girls	64	125	122	136	109	89	108	109	107	143	174	142	107	51	1586
			Both	132	228	262	264	254	214	221	199	231	286	342	271	207	79	3190

Table 3.—*Number of DMF teeth per child (permanent dentition)*

Locality	Residence history	Water source	Sex	Age at survey (years)													
				6	7	8	9	10	11	12	13	14	15	16	17	18	All*
Non-fluoride	Born and reared	City	Boys	0.0	2.0	1.8	2.8	3.1	2.7	4.6	6.8	9.1	8.8	11.8	6.8	16.7	5.9
			Girls	0.4	1.5	2.5	2.3	3.1	6.6	5.6	7.8	9.8	9.8	12.4	11.9	12.5	6.6
			Both†	0.2	1.8	2.2	2.6	3.1	4.7	5.1	7.3	9.5	9.3	12.1	9.4	14.6	6.3
		Home well	Boys	0.1	2.0	1.6	2.4	2.8	3.2	5.3	6.6	8.3	8.3	9.9	9.4	8.6	5.3
			Girls	1.1	1.0	3.0	3.3	4.4	7.2	7.3	9.0	8.2	11.5	11.7	15.5	10.8	7.2
			Both†	0.6	1.5	2.3	2.9	3.6	5.2	6.3	7.8	8.3	9.9	10.8	12.5	10.2	6.3
	Migrants	City	Boys	0.4	0.6	1.8	1.9	2.5	2.9	3.9	6.1	7.5	7.6	9.4	9.4	9.3	4.9
			Girls	0.3	1.0	1.6	2.5	3.4	3.7	3.9	8.8	7.5	8.1	11.2	10.4	13.3	5.8
			Both†	0.4	0.8	1.7	2.2	3.0	3.3	3.9	7.5	7.5	7.9	10.3	9.9	11.3	5.4
Fluoride	Born and reared	City	Boys	0.0	0.1	0.2	0.4	0.7	0.6	1.2	1.9	1.3	2.3	2.8	4.8	5.4	1.7
			Girls	0.1	0.2	0.4	0.7	0.7	0.9	1.5	2.0	3.0	3.0	4.4	5.0	3.8	2.0
			Both†	0.1	0.2	0.3	0.6	0.7	0.8	1.4	2.0	2.2	2.7	3.6	4.9	4.6	1.9
		Home well	Boys	0.0	1.6	2.2	1.1	2.1	1.5	2.5	6.4	7.0	7.0	7.2	8.3	7.3	4.2
			Girls	0.0	0.9	1.5	1.1	1.8	3.1	4.4	5.5	7.0	7.5	8.5	6.6	3.3	4.5
			Both†	0.0	1.3	1.9	1.1	2.0	2.3	3.5	5.9	7.0	7.3	7.9	7.5	5.3	4.4
	Migrants	City	Boys	0.2	0.2	0.4	1.4	1.1	2.2	2.2	3.9	4.9	7.2	6.9	6.5	6.7	3.4
			Girls	0.2	0.5	1.6	1.8	1.9	2.4	3.5	4.7	6.8	7.2	8.9	7.8	8.5	4.3
			Both†	0.2	0.4	1.0	1.6	1.5	2.3	2.8	4.3	5.9	7.2	7.9	7.2	7.6	3.9

* Simple arithmetic mean of age-specific rates.
† Simple arithmetic mean of sex-specific rates.

children who were arranged in age groups of single years.

FINDINGS

Deciduous Teeth of Lifetime Residents.—Findings on caries experience in the deciduous teeth of children residing in the fluoride or nonfluoride areas are shown in table 2 and figure 1. The deciduous teeth are more protected against caries among children to whom the fluoride city water is available than among children who use farm well water, even though their homes are in the fluoride area. It is to be noted also that among children to whom city water is available, those receiving the fluoride water are protected significantly more against caries in the deciduous teeth than children receiving nonfluoride city water. At 5 years of age, children receiving nonfluoride city water average more than 18 decayed or filled deciduous teeth per 100 deciduous teeth present, whereas children of the same age to whom the fluoride city water is available average less than 3, a rate nearly 85 percent below that of the children exposed to the nonfluoride water. At every age beyond 5 years and until the deciduous teeth are exfoliated (at about 12 years of age), the children of the fluoride area who drink city water average a lower caries rate in the deciduous teeth. It is to be emphasized that toward the end of the life of the deciduous teeth (at about 12 years of age) the attack rate for children in the fluoride area is about half that of children in the nonfluoride area.

Permanent Teeth of Lifetime Residents.—Data which give findings on the number of DMF teeth per child born and reared in the fluoride or in the nonfluoride areas are given in table 3. These data are shown graphically in figure 2. Children residing in the fluoride area who were born and reared therein and consumed the city water have, at every age, a lower DMF rate than do children consuming the city water in the nonfluoride area. With advancing age the absolute difference between the two groups widens. However, the relative differences decrease with advancing age. At 7 years of age the children of the fluoride area have a DMF rate nearly 90 percent lower than the children of the nonfluoride area; at 10 years of age the former have a rate about 77 percent lower than the nonfluoride children; and at age 15 they are 71 percent lower. For all ages considered together among children born and reared in one area or the other, those consuming the fluoride water have an average DMF rate nearly 70 percent lower than do the children taking the nonfluoride water.

Home Wells versus City Water.—Of the 1,285 children born and reared in the fluoride area, 341, or approximately 26 percent, received their drinking water at home from farm wells. Those children obtained city water while attending school in the fluoride area.

As shown by the data given in table 3 and figure 2, the children drinking farm well water in the fluoride area are not protected against caries to the same extent as are the children consuming the city water. However, they experience some benefit (presumably by drinking the fluorine water during school attendance) because their caries rates are lower than those of children exposed to city water in the nonfluoride area.

Among the 598 children born and reared in the nonfluoride area, 258 or about 43 percent were from homes where the water was obtained from individual farm wells. As shown by the data given in table 3 and figure 2, the caries rates of such children do not differ significantly from those of children consuming the city water in the same area.

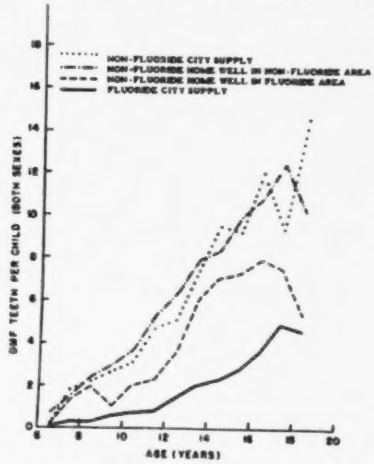

Figure 2.—*Relation between age and number of DMF teeth per child born and reared in specified area.*

Deciduous Teeth of Migrants.—Findings on the deciduous teeth of migrants into the two areas are shown in table 4 and are summarized in figure 3. Among migrants into the fluoride area 5 to 9 years of age at the time of survey, those in the area the shortest time (0 to 4 years) average 26.0 carious or filled deciduous teeth per 100 deciduous teeth present; whereas children of the same age resident in the area the longest time (5 to 9 years) average about half that rate (13.6). Among migrants to the fluoride area 10 to 14 years of age, those who have been residents 0 to 4 years average nearly 51 attacked deciduous teeth per 100 deciduous teeth present, whereas those resident 5 to 9 years average less than 36 attacked deciduous teeth per 100. It should be pointed out that the protective effect of exposure is more pronounced for the younger children, resulting in a relative difference of the order of 50 percent in the caries attack rate between the longest and shortest exposure for 5 to 9 year old children, whereas the corresponding difference for the older children is of the order of 30 percent; that is, the sooner a child born outside the fluoride area takes residence in the area, the lower the caries attack rate in the deciduous teeth.

Children 5 to 9 years old at the time of survey and born and reared in the fluoride area (and therefore having a lifetime exposure) average 11.1 attacked deciduous teeth per 100 deciduous teeth present, as contrasted with the corresponding rate of 13.6 for children exposed nearly all their lives (5 to 9 years). Among children 10 to 14 years of age at the time of survey, those exposed all their lives in the fluoride area average 26.7 attacked deciduous teeth, while the corresponding rate for children of the same ages exposed 5 to 9 years is 35.4. These findings suggest that a lifetime exposure in the fluoride area perhaps provides only a slightly higher degree of protection against caries than exposure begun shortly after birth (fig. 3).

For migrants into the nonfluoride area, the effects of exposure to the drinking water also are evident in the deciduous teeth, and this suggests the presence of noxious factors in that water. The findings on migrants 5 to 9 years of age at the time of the survey are not consistent. However, for migrants 10 to 14 years of age at the time of the survey, those exposed the longest time, 5 to 9 years, have nearly 66 carious or filled deciduous teeth per 100 deciduous teeth present, whereas migrants of the same age exposed only 0 to 4 years average fewer than 46 attacked deciduous teeth per 100 deciduous teeth present.

Permanent Teeth of Migrants.—By referring again to table 3 and then to figure 4, it becomes clear that migrants into the fluoride area who consume the city water have DMF prevalence rates consistently higher at every age than children also taking the same water but born and reared in the same area. But the caries prevalence rates of these migrants are consistently below those of children born and reared in the nonfluoride area. On the other hand migrants into the nonfluoride area have rates consistently higher than migrants into the fluoride area. For all ages considered together, the migrants into the fluoride area average 3.9 DMF teeth per child. For contrast the corresponding rate for children born and reared in the fluoride area (and drinking city water) is 1.9 and for native-born children of the nonfluoride area 6.3 DMF teeth.

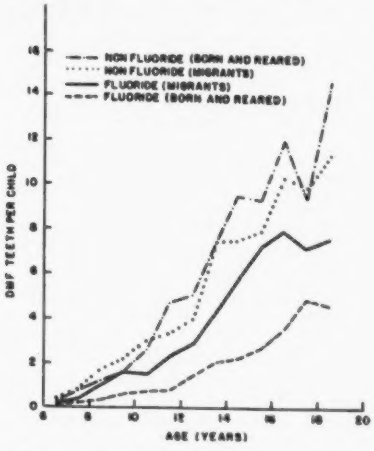

FIGURE 4.—*Relation between age and number of DMF teeth per child.*

TABLE 4.—*Number of deciduous teeth, carious or filled, per 100 deciduous teeth for migrants into specified areas*

Age Group	Area	Sex	Duration of exposure (years)									
			0	1	2	3	4	5	6	7	8	9
5–9	Fluoride	Boys	24.3	26.2	24.4	24.8	20.3	18.3	11.1	19.1	22.4	------
		Girls	22.1	17.3	27.2	20.4	37.0	10.1	18.1	14.7	0.0	11.1
		Both†	23.2	21.8	25.8	22.6	28.7	14.2	14.6	16.9	11.2	11.1‡
	Nonfluoride	Boys	29.6	17.5	16.7	32.6	49.2	28.8	35.7	------	------	------
		Girls	20.0	34.8	57.1	30.4	21.4	29.4	66.7	------	12.5	------
		Both†	24.8	26.2	36.9	31.5	35.3	29.1	51.2	------	12.5‡	------
10–14	Fluoride	Boys	83.3	50.0	47.6	32.8	36.2	27.7	25.5	27.3	50.0	6.7
		Girls	100.0	35.7	55.9	------	33.3	34.6	45.5	28.0	19.4	88.9
		Both†	91.7	42.9	51.8	32.8‡	34.8	31.2	35.5	27.7	34.7	47.8
	Nonfluoride	Boys	47.4	0.0	100.0	33.3	95.2	33.3	25.0	68.8	45.5	100.0
		Girls	------	0.0	0.0	------	------	------	100.0	------	63.6	------
		Both†	47.4‡	0.0	50.0	33.3‡	95.2‡	33.3‡	62.5	68.8‡	54.6	100.0‡
All	Fluoride	Boys	53.8	38.1	36.0	28.8	28.3	23.0	18.3	23.2	36.2	6.7*
		Girls	61.1	26.5	41.6	20.4	35.2	22.4	31.8	21.4	9.7	50.0
		Both†	57.5	32.3	38.8	24.6	31.8	22.7	25.1	22.3	23.0	28.4
	Nonfluoride	Boys	38.5	8.8	58.4	32.0	72.2	30.7	30.4	68.8*	45.5*	100.0*
		Girls	20.0*	17.4	28.6	30.4*	21.4*	29.4*	83.4	------	38.1	100.0*
		Both†	29.3	13.1	43.5	31.2	46.8	30.1	56.9	68.3‡	41.8	100.0

* Findings based on only one age group.
† Simple arithmetic mean of sex-specific rates.
‡ Findings based on only one sex.

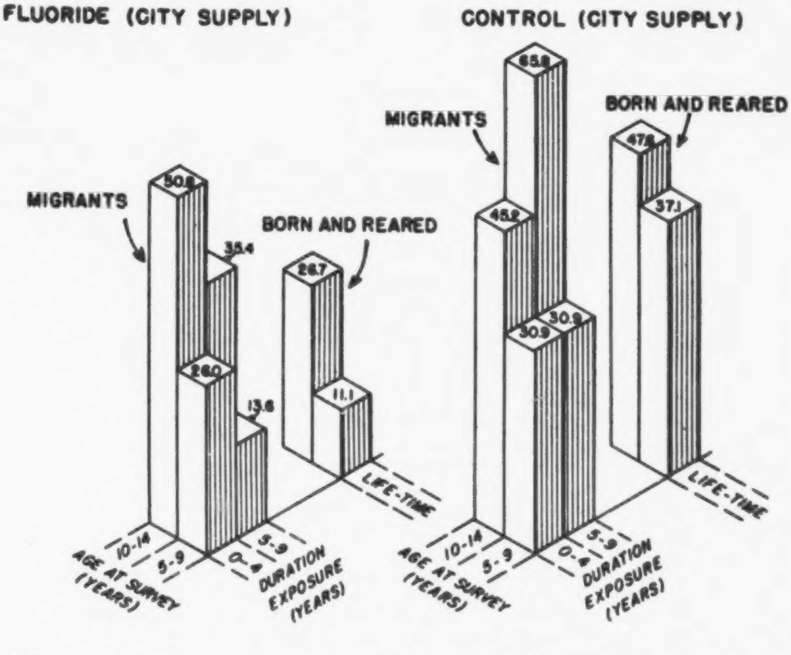

FIGURE 3.—*Relation between number of carious and filled deciduous teeth per 100 deciduous teeth present and duration of exposure in specified areas.*

As shown graphically in figure 5, which was plotted from the material given in table 5, children coming into the fluoride area, 5 to 9 years of age at the time of survey, who have been exposed to the fluoride city water for 5 to 9 years average 5.5 DMF teeth per 100 person-years of age. All rates given previously have been prevalence rates, which represent the amount of caries experience accumulated up to given ages. The present rate is an incidence rate which represents the amount of caries experienced per 100 person-years of age over the span of years up to the specified ages. On the other hand, migrants of the same ages exposed only 0 to 4 years show a caries incidence rate more than a third higher (8.6 DMF teeth per 100 person-years of age). For migrants 10 to 14 years of age at the time of survey, those who have been in the fluoride area the shortest time (0 to 4 years) average the highest incidence rate (36.7), and those in the area the longest time (10 to 14 years) average the lowest rate (15.5). For older migrants 15 to 19 years of age at the time of survey, those in the area the longest time (10 to 14 years) have the lowest rate (33.3), whereas those in the area the shortest time (0 to 4 years) have the highest rate (56.6). These several findings may be interpreted to indicate that children do not need to be born in the fluoride area in order to experience protection against caries attack, but the sooner they arrive in the area after they are born the more they are protected.

As indicated by other material shown in table 5 and figure 5, a strikingly different picture is presented by migrants into the nonfluoride area. The findings are such as to suggest that just as there are inhibitory factors in drinking water which decrease the rate of caries attack, there may be accelerating factors in other drinking waters which increase that rate.

For migrants coming into the nonfluoride area and to whom the city water supply is available, those who have been in the area the shortest time have the best teeth, while those who have stayed the longest time have the worst teeth. As shown by the data in table 5 and figure 5 for migrants into the nonfluoride area 10 to 14 years of age at the time of survey, those in the area 0 to 4 years average 37.6 DMF teeth per 100 person-years of age, those in the area 5 to 9 years average 44.3, while migrants of the same age who have lived in the area 10 to 14 years average the most DMF teeth per 100 person-years of age (57.5). Among migrants to the nonfluoride area 15 to 19 years of age at the time of survey, those in the area the shortest time have the best teeth (49.6 DMF teeth per 100 person-years of age) and those in the area the longest time have the worst teeth (70.0 DMF teeth per 100 person-years of age) for the age-group.

The contrast between the favorable effect of the fluoride area on the one hand and the deleterious character of the nonfluoride area on the other for migrants and for lifetime residents is summarized in figure 6 for 10 to 14 year old children. It can be noted that children who have migrated into the fluoride area and have been there the longest time have the lowest attack rate (15.5), a rate only a little higher than that of lifetime residents (11.5) of the same age, while the children who have migrated to the nonfluoride area and have been there the longest time have the highest caries attack rate (57.2), a rate only a little higher than that of lifetime residents (50.1) of the same ages.

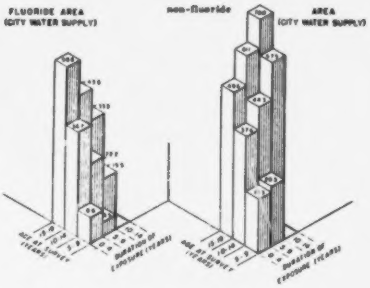

FIGURE 5.—*Number of DMF teeth per 100 person-years of age among migrants to specified areas by duration of exposure in the areas and by age at time of survey.*

DISCUSSION

The findings given in the present report provide several facets of new information regarding the inhibiting effects of fluoride-bearing waters on dental caries. Additional information derived from a nonfluoride area in close geographic proximity raises the question whether other waters may bear principles which favor caries attack.

Examination of both deciduous and permanent teeth of children of school ages born and reared in the fluoride area indicates clearly that residence in such an area in New Jersey is associated with low caries attack rates in the teeth of both the deciduous and permanent dentitions.

It is clear that children need not be born in the fluoride area to experience the protection associated with residence in that area. The earlier in age they arrive and the longer they are exposed, the greater is the protection they experience.

The implications of these findings are apparent for other localities where the addition of fluorides to city water supplies now is being accomplished or planned. For areas where fluorination has been started, it would be reasonable to predict that favorable effects will appear and be measurable in both the deciduous and permanent teeth of preschool children and in children of the early elementary grades within two years after fluorination is begun.

It has been shown (*6–8*) that dental caries is a family matter; that is, parents with the highest DMF rates for their age have children who show the highest rates, whereas parents with the lowest rates have children who average the lowest rates for their ages. Such findings suggest that familial susceptibility exists in dental caries. This factor may be dominant in determining the ultimate level of DMF experience when adulthood is reached. However, the findings described here make it clear that a trace element in drinking water can superimpose its influence and affect the DMF rate.

These considerations apply perhaps equally to the opposite findings encountered in the nonfluoride area where the earlier the migrants arrive and the longer they stay the greater becomes the intensity of attack by dental caries. Clinical examination of the children exposed to the nonfluoride waters and findings on the individual teeth suggest that there may be something in the water which favors the dissolution of the calcium salts in the enamel. These latter findings (to be published in a later report) show that the upper anterior teeth of children in the nonfluoride area are profoundly involved with caries. A large proportion of the children of high school age show large black and yellow stained crescent-shaped cavities on all proximal surfaces of all the six upper anterior teeth and, in many instances, in the lower anterior teeth as well.

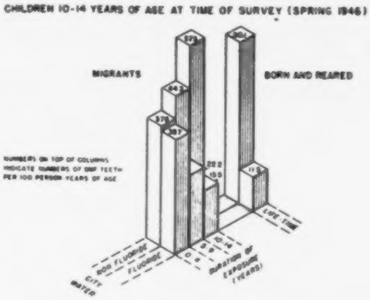

FIGURE 6.—*Number of DMF teeth per 100 person-years of age by duration of exposure for children of specified ages and specified areas.*

CONCLUSION

The findings described here may be viewed as proof that fluorination (accidental) of a community water supply with 1.2 to 2.2 p.p.m. of naturally occurring fluoride salts results in a marked

TABLE 5.—*Number of DMF teeth per 100 person-years of age for migrants into specified areas*

Age group	Sex	Area (city water)	Duration of exposure (years)														
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
5-9	Boys	Fluoride	10.8	4.4	8.3	5.3	5.8	2.2	20.8	0.0	0.0	—	—	—	—	—	—
		Nonfluoride	12.7	29.0	11.1	14.3	10.3	17.9	28.6	—	—	—	—	—	—	—	—
	Girls	Fluoride	12.7	8.9	14.6	6.2	9.0	6.0	8.9	5.6	0.0	0.0	—	—	—	—	—
		Nonfluoride	14.7	34.8	30.4	17.7	19.0	12.5	22.2	—	11.1	—	—	—	—	—	—
	Both [1]	Fluoride	11.8	6.7	11.5	5.8	7.4	4.1	14.9	2.8	0.0	—	—	—	—	—	—
		Nonfluoride	13.7	31.4	20.8	16.0	14.7	15.2	25.4	—	—	—	—	—	—	—	—
10-14	Boys	Fluoride	31.7	37.7	24.5	28.3	24.8	33.1	24.1	16.2	14.4	17.4	12.5	6.3	4.8	—	—
		Nonfluoride	47.6	37.3	33.3	38.5	22.4	11.1	23.5	43.2	42.4	67.7	92.3	16.7	84.6	—	—
	Girls	Fluoride	60.0	39.7	31.1	54.4	34.4	24.2	26.3	20.2	18.0	28.4	31.3	23.7	14.5	14.3	0.0
		Nonfluoride	22.2	47.6	55.8	—	37.1	61.5	18.2	55.4	34.8	84.6	20.8	100.0	—	53.8	—
	Both [1]	Fluoride	45.9	38.7	27.8	41.4	29.6	28.7	25.2	18.2	16.2	22.9	21.9	15.0	9.7	—	—
		Nonfluoride	33.4	42.5	44.6	—	29.8	36.3	20.9	49.3	38.6	76.2	56.6	58.4	—	—	—
15-19	Boys	Fluoride	52.2	125.0	48.6	28.1	50.0	48.3	30.8	28.4	57.7	28.6	14.9	41.2	23.3	22.7	29.6
		Nonfluoride	50.0	56.2	—	42.2	44.9	98.9	42.4	—	31.2	—	47.1	100.0	—	64.5	—
	Girls	Fluoride	50.0	43.5	56.3	57.0	55.1	58.7	51.9	43.7	40.0	46.9	46.3	45.9	27.1	25.3	56.1
		Nonfluoride	—	87.5	33.3	53.1	13.2	—	—	73.8	—	—	100.0	—	6.2	68.3	50.0
	Both [1]	Fluoride	51.1	84.3	52.5	42.6	52.6	53.5	41.4	36.1	48.9	37.8	30.6	43.6	25.2	24.0	46.9
		Nonfluoride	—	71.9	—	47.7	29.1	—	—	—	—	—	73.6	—	—	66.4	—
All	All [2]	Fluoride	36.3	43.2	30.6	29.9	29.9	25.4	27.2	19.0	21.7	30.4	26.3	29.1	17.5	24.0	42.9
		Nonfluoride	23.4	48.6	32.7	31.9	24.5	25.8	23.2	49.3	38.6	76.2	65.1	58.4	—	66.4	—

[1] Simple arithmetic mean of sex-specific rates.

[2] Simple arithmetic mean of age-specific rates.

reduction in the incidence and prevalence of dental decay in persons born in or migrating to the area where such waters are consumed. The findings are based on persons exposed as long as nineteen years to the fluoride waters.

It should be pointed out with some emphasis that no observations of a general medical nature were made in the area. Hence a complete elucidation of the general systemic benefits or disadvantages of a fluorinated water cannot be given here. However, it is necessary to recognize that the New Jersey area provides the opportunity for assaying the general medical effects, a problem which is actively under consideration in several areas where sodium fluoride is being added to community water supplies. Significant findings in these directions may be anticipated through medical observations which now may be made after nineteen years of exposure of a population to fluoride-bearing waters in New Jersey.

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(*1*) Fluorine in Dental Public Health. Symposium Conference of the New York Institute of Clinical Oral Pathology, October 30, 1944. New York.

(*2*) Klein, Henry: Do Certain Drinking Waters Favor Dental Caries? Science *105*: 4 (Jan. 3) 1947).

(*3*) All children were examined by the author and Dr. John F. Cody, senior assistant dental surgeon, USPHS. The findings were recorded with the assistance of Mrs. Russell T. Atkinson.

(*4*) The symbol, DMF, first proposed in 1937 (*7*) and now widely used indicates decayed, missing and filled permanent teeth.

(*5*) Klein, Henry, and Palmer, C. E.: Dental Caries in American Indian Children. Public Health Bulletin No. 239. Washington, D.C.: U.S. Government Printing Office (Dec.) 1937.

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DENTAL EFFECTS OF COMMUNITY WATERS ACCIDENTALLY FLUORINATED FOR NINETEEN YEARS. II. Differences in the Extent of Caries Reduction Among the Different Types of Permanent Teeth

By HENRY KLEIN

In preceding reports it was shown that children, born and reared in a New Jersey area, and who drink waters naturally containing from 1.3 to 2.2 parts per million of fluoride have, as a group, a lower than usual prevalence of dental caries experience (*1*, *2*). However, the children are not protected all to the same extent. Those who have the least amount of caries experience have parents showing smaller than average amounts of experience with dental disease while the children who have the most caries experience (in spite of their residence in a fluoride environment) are the ones whose parents have had more than average amounts of such experience. Hence a powerful familial factor in dental caries susceptibility (*3*) is evident even in an area where fluoride is available in the community water supplies (*4*). It follows therefore, that variation exists among different children in their response to the caries-inhibitory effects of fluoride waters. Certain children are protected more, others less, and this variation is associated, among other factors, with familial susceptibility (*5*).

It has been demonstrated also that there exists among the different kinds of teeth (incisors, canines, premolars, and molars) different degrees of what might be termed, characteristic susceptibility to dental decay (*6*). Hence, the molars are most frequently attacked by caries while the canines are the least often involved. To what extent are these typical or expected susceptibilities of the different teeth (determined in nonfluo-

Table 1.—*Number of white children (both sexes) examined*

Item specified	Age (years)															
	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	All
Fluoride:																
Lifetime	71	73	97	84	83	65	53	51	60	53	61	63	39	15	6	874
Migrants	21	57	52	56	48	53	72	50	59	73	65	49	43	21	6	725
Nonfluoride:																
Hagerstown (migrants and lifetime)	......	327	403	487	493	529	531	596	565	695	651	445	355	148	32	6,257
Williamstown-Clayton (lifetime)	10	28	30	43	41	24	21	26	32	23	27	15	15	5	......	340

ride areas) influenced or affected by fluoride exposure? Are all the teeth protected to the same extent or are some teeth protected more than others?

Analysis of this, and related questions, reveals that the various morphological types of teeth in the permanent dentition are indeed affected differently by exposure to fluoride. Certain teeth, particularly those located in the anterior of the mouth, are protected most while the molars, which are placed most posterior in the mouth, are protected least.

FINDINGS

The study to be reported here is based on examination of nearly 2,000 children residing in New Jersey and more than 6,000 children of Hagerstown, Md. Of the total of New Jersey children ranging in age from 5 to 19 years, 874 were born and reared in a fluoride area (lifetime residents), 725 moved into the fluoride area at various ages subsequent to birth in places outside the fluoride areas (migrants) and 340 children were lifetime residents of the non-fluoride area (table 1).

Each child was examined under good light with mirror and explorer. The number of DMF teeth per 100 children of particular ages for each of the pairs of the seven kinds of teeth in the mandible and maxilla (excluding the third molars) are shown in table 2 for lifetime residents and for migrants in the fluoride area. The symbol "DMF" first introduced by the author in the year 1937 (*7*) and now widely used indicates decayed, missing and filled permanent teeth. For comparison corresponding DMF rates of children of the same ages are given in the same table for two nonfluoride areas: Hagerstown, Md., and Williamstown and Clayton, N.J.

Dr. John F. Cody, dental officer, Public Health Service, made all the examinations reported; both those in Hagerstown, Md. and those in New Jersey.

Williamstown and Clayton were originally selected as the comparison areas (nonfluoride). However, as shown in table 2 and elsewhere (*8*), the Williamstown-Clayton children have excessively high DMF rates and therefore cannot be considered as representative of a nonfluoride area especially since evidence is available (*8*) to suggest that the Williamstown-Clayton waters contain principles which may accelerate caries attack. However, the DMF rates for Hagerstown children agree rather closely with those obtained for San Francisco (*9*) and New York City children (*10*). Hence, the Hagerstown DMF rates may be tentatively accepted as representative of nonfluoride areas and are used here for comparison with the fluoride area in New Jersey.

Lifetime exposure to fluoride.—As shown in table 2, the 5- to 9-year-old-lifetime residents of the fluoride area average about 17 DMF teeth per 100 children. The migrants of the same ages average three times as many DMF teeth while children of the same ages in Hagerstown average about five times as many more DMF teeth as do the lifetime residents of the fluoride area. Inspection of the data given in table 2, and study of figures 1 and 2, reveal that (with but a few exceptions), for each kind of tooth at each age level, the DMF rates of the migrants are lower than those of Hagerstown children, while the DMF rates of the lifetime residents are in turn lower than the corresponding rates of the migrants. The Williamstown-Clayton children have the highest DMF rates for each kind of tooth (table 2).

Table 2.—*Number of DMF teeth per 100 white children (both sexes), by age groups*

Item specified	Age	Maxillary teeth left and right sides							Mandibular teeth left and right sides							All [1]
		CI	LI	C	PM₁	PM₂	M₁	M₂	CI	LI	C	PM₁	PM₂	M₁	M₂	
Fluoride: Lifetime	5–9	1.5	0	0	0	0	8.3	0	0	0	0	0	0	7.2	0	17.0
	10–14	.7	1.8	1.8	4.6	3.2	36.5	5.3	0	0	0	1.8	3.2	50.9	13.1	13[illegible].9
	15–19	7.1	8.2	2.2	10.3	16.3	68.5	39.7	.5	0	0	4.9	10.9	11.9	79.3	35[illegible].7
Migrants	5–9	0	0	0	0	0	19.2	0	0	0	0	0	.4	32.1	0	51.7
	10–14	9.1	10.4	0	6.2	6.8	96.1	13.7	1.0	.3	.3	2.0	7.5	119.5	32.6	305.9
	15–19	36.4	33.2	6.0	30.4	31.5	124.5	58.2	2.2	1.6	1.6	10.3	28.3	142.4	87.0	593.5
Nonfluoride: Hagerstown (lifetime and migrants)	5–9	2.2	1.8	.1	.8	.4	28.4	0	.4	.2	0	.2	.6	49.6	0	84.7
	10–14	25.2	23.2	1.4	11.3	11.2	106.0	15.0	6.2	2.4	.2	2.4	9.2	139.6	33.6	386.9
	15–19	47.6	52.8	11.4	41.6	45.4	141.0	87.0	6.8	6.0	2.4	11.2	30.9	169.8	119.2	782.0
Williamstown-Clayton (lifetime)	5–9	1.3	1.3	0	1.3	1.3	64.5	0	1.3	1.3	0	.7	.7	95.4	.7	169.8
	10–14	42.1	42.9	5.6	18.3	22.2	140.5	36.5	9.5	8.7	2.4	7.1	20.6	160.3	81.0	597.7
	15–19	82.3	75.8	24.2	75.8	82.3	164.5	116.1	11.3	9.7	3.2	24.2	66.1	183.9	150.0	1,069.4

[1] Summation of the individual DMF teeth to give total DMF teeth of all kinds per 100 children of given age groups.

Equally evident is the finding that all the different kinds of teeth are not protected by the fluoride waters to the same extent. The discussion at this point will deal only with findings on 15- to 19-year-old children who are lifetime residents as compared with the findings for children of the same ages, in Hagerstown, and in Williamstown and Clayton. Hagerstown children average nearly 48 DMF upper central incisors in contrast to only 7 DMF upper central incisors among the lifetime residents of the fluoride area. This is approximately a sevenfold difference. As between the two groups, the upper lateral incisors show more than a sixfold difference, the upper canines a fivefold difference, the upper first premolars a fourfold difference and the upper second premolars a threefold difference while the amount of caries experienced in the upper molars as between the two areas, represents approximately a twofold difference for 15- to 19-year-old children.

Using Williamstown-Clayton for comparison with the fluoride area it is found that the difference in the DMF rates for the upper central incisors is nearly twelvefold, for the upper laterals the difference is ninefold, for the upper canines it is twelvefold, the upper first premolar difference is sevenfold, the upper second premolar is fivefold, the upper first molar is more than twofold and the upper second molar is more than twofold for 15-to 19-year-old children.

In the lower jaw the differences between Hagerstown and the New Jersey fluoride lifetime residents 15 to 19 years of age represents a more than tenfold difference for the central and lateral incisors and canines, about a twofold difference for the first premolars, more than a threefold difference for the second premolars but less than a twofold difference for the molars.

If the comparison is made in terms of the New Jersey fluoride lifetime residents 15 to 19 years of age and children of the same ages who are lifetime residents of the nonfluoride area in New

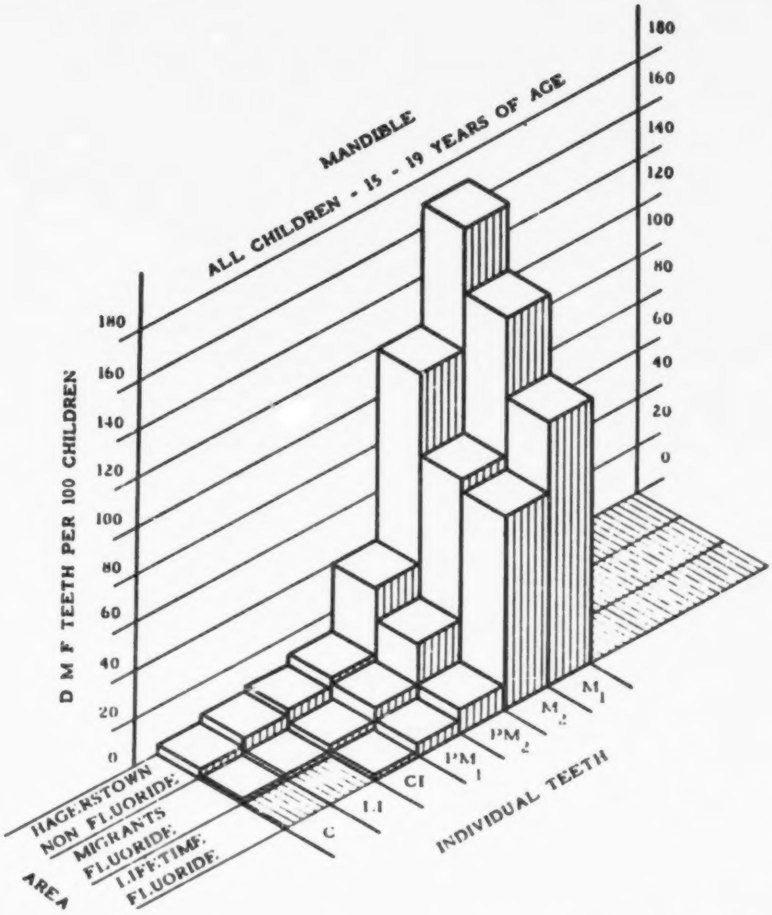

FIGURE 1.—*Number of DMF teeth of specified kinds, left and right sides of the lower jaw (mandible) per 100 children of both sexes, 15–19 years of age; by specified areas and residence history. CI—central incisor, LI—lateral incisor, C—canine, PM—first premolar, PM_2—second premolar, M—first molar, M_2—second molar.*

Jersey (Williamstown and Clayton), the differences between the DMF rates for the lower teeth are as follows: Central incisors, twenty-twofold; first premolars, nearly fivefold; second premolars, sixfold; first molars, 1.7 times and the second molars, 1.9 times.

From these findings, it becomes clear that exposure to fluoride waters over a life time of 15–19 years will reduce the amount of caries experience in each type of tooth; but to a different extent for the various types. The DMF experience of the molars is affected least while that of the upper and lower incisors and canines is affected most. Observations on DMF experience in the upper anterior teeth have been reported by other workers (*11, 12*). These differences between the DMF experience rates of the individual pairs of teeth as between Hagerstown and lifetime residents of the fluoride area can be expressed also as the absolute difference, and as the percent reduction below the Hagerstown rates as shown in table 3 and figure 3. Hence, among lifetime residents of the fluoride area, caries experience is reduced 100 percent below the expected rate (Hagerstown) in the lower lateral incisors and canines. It is necessary to point out here that, where the reduction in the number of DMF teeth is 100 percent, this fact does not indicate an absolute protective power of fluoride waters but rather reflects sampling fluctuations due to a relatively small number of cases. There is a nearly 93-percent reduction for the lower central incisors, and approximately an 85-percent reduction for the upper central and lateral incisors, an 80-percent reduction for the upper canines, more than a 70-percent reduction for the upper first and lower second premolars, a 64-percent reduction for the upper first premolars. The smallest reduction below the Hagerstown rate is shown by the lower first and second molars in which about a 33-percent reduction is achieved by a lifetime exposure in the fluoride area. The percentage reduction in the DMF rates of the lifetime residents in the fluoride versus the nonfluoride areas of New Jersey are also shown in table 3.

Various durations of exposure to fluoride.—Analyses of DMF findings on migrants exposed in a fluoride area may be viewed as providing information from which some of the effects of artificial fluorination may be anticipated. Migrants establishing residence in a fluoride area and consuming the waters containing fluoride may be considered perhaps as the equivalent of persons residing in a non-fluoride area but to whom subsequently fluoride exposure is provided by artificial fluorination of their water supplies.

As shown previously, the teeth of migrants are protected against caries by the fluoride waters. It has also been shown that the extent to which caries incidence among migrants is depressed is, among others variables, a function of (*a*) their age at time of arrival in the area and (*b*) the length of time they stay in the area (*1, 8*).

The question at this point in the discussion deals more specifically with the effect of duration of exposure in a fluoride area on the caries experience of the individual types of teeth among the migrants. Material bearing on that question is shown in table 4 and in figures 4 and 5.

From these data, it becomes evident that among children migrating to and establishing residence within a fluoride area of New Jersey, certain teeth are protected against caries attack more than are other teeth. It is clear that the children arriving at the earliest ages and exposed the longest time (and therefore the teeth earliest and longest exposed) have significantly lower amounts of DMF experience than do the children (and their individual types of teeth) most recently arrived and shortest exposed in the area.

As was true of life-time residents in the fluoride area, so with the migrants, caries experience is least retarded in the lower molar teeth by exposure in the fluoride area, while the upper and lower anterior teeth are most protected. The extent to which caries experience is reduced in the different teeth is determined more or less quantitatively by duration of exposure and age at first arrival in the area (figs. 4 and 5). Computations show that as between the longest and shortest exposed among the migrants, caries experience is reduced 100 percent in the upper and lower anterior teeth (incisors and canines), approximately 86 percent in the upper first premolars, about 66 percent in the upper first molars and a little more than 50 percent in the lower first molars.

The values derived here for the percentage reduction in the DMF rates of different teeth should not be accepted as firmly established predictable values. As shown in the present report, the percentage reduction values are based on Hagerstown DMF rates which are accepted only tentatively as representative of nonfluoride areas. Accordingly, the data presented here are meant only to serve as illustrations of the findings that fluoride exposure does not protect all the different teeth to the same extent, but rather provides a greater effect on anterior teeth than on teeth located more posterior in the mouth.

COMMENT

It is now generally accepted that children born and reared in an area where they consume waters containing 1 to 2 parts per million of naturally occurring fluoride have, as a group, a lower prevalence of caries experience than children born and reared in a nonfluoride area such as Hagerstown, Md. Furthermore, children not born in a fluoride area, but migrating and establishing residence therein are also protected against caries attack; but to an extent which is determined more or less quantitatively by age at time of arrival, and the length of time they are resident in the fluoride area (*1, 8*).

The depressant effect of fluorides on caries may be approximately of equal potential for all the different types of teeth. However, because of the different gradients in the characteristic or expected caries susceptibilities of the different teeth, the resultant effect may be a variation in the percent reduction of caries among the different teeth, such as described above for the New Jersey children. Consideration of the differences in caries reduction among the different teeth leads to the view that the differential effects are perhaps related to basic differences in the characteristic susceptibilities of the different teeth. By this speculation, it would follow that the tooth most protected by exposure to fluoride would be that tooth having the strongest natural resistance to caries attack; the tooth least protected would be the tooth having the strongest natural tendency to be attacked by caries.

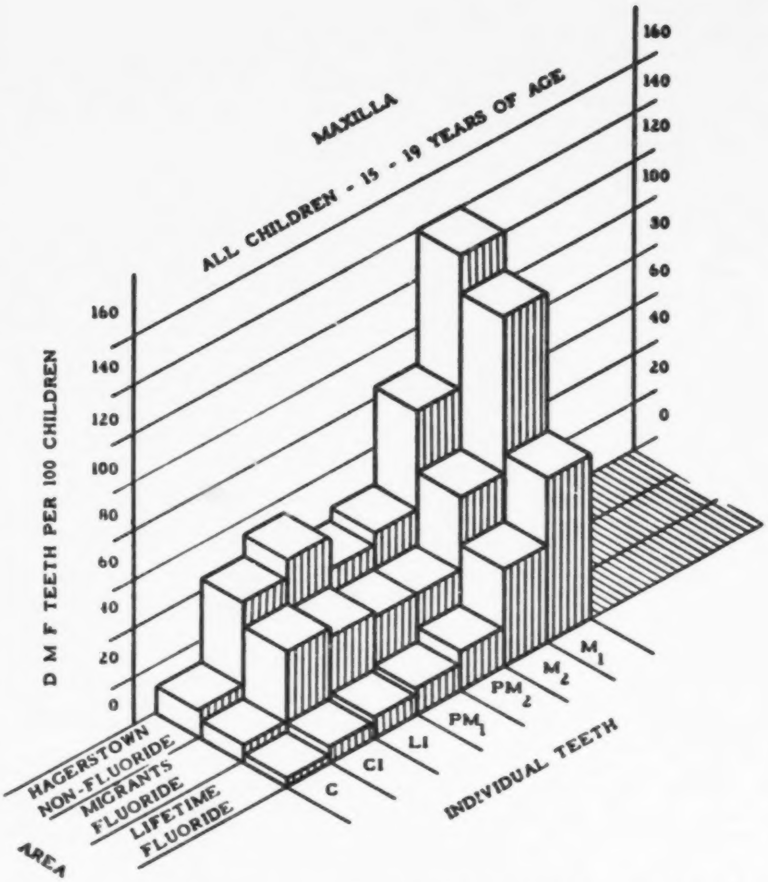

FIGURE 2.—*Number of DMF teeth of specified kinds, left and right sides of the upper jaw (maxilla) per 100 children of both sexes, 15–19 years of age; by specified areas and residence history. CI—central incisor, LI—lateral incisor, C—canine, PM—first premolar, PM_2—second premolar, M—first molar, M_2—second molar.*

TABLE 3.—*Absolute differences and percent reduction in the DMF rates for 15–19 year-old children (both sexes)*

ABSOLUTE DIFFERENCE IN DMF RATES

Item specified	Maxillary teeth left and right sides							Mandibular teeth left and right sides						
	CI	LI	C	PM_1	PM_2	M_1	M_2	CI	LI	C	PM_1	PM_2	M_1	M.
Between Williamstown-Clayton (nonfluoride) and the fluoride areas in New Jersey	75.2	67.6	22.0	65.5	66.2	96.0	76.4	10.8	9.7	3.2	19.3	55.2	73.0	70.7
Between Hagerstown and the fluoride areas in New Jersey	40.5	44.6	9.2	31.3	29.1	72.5	47.3	6.3	6.0	2.4	6.3	28.9	58.9	39.9

PERCENT REDUCTION IN DMF RATES

Item specified	CI	LI	C	PM_1	PM_2	M_1	M_2	CI	LI	C	PM_1	PM_2	M_1	M.
Between Williamstown-Clayton (nonfluoride) and the fluoride areas in New Jersey	91.4	80.2	90.9	86.4	80.4	58.4	65.8	95.6	100.0	100.0	79.8	83.5	39.7	47.1
Between Hagerstown and the fluoride areas in New Jersey	85.1	84.5	80.7	75.2	64.1	51.4	54.3	92.6	100.0	100.0	56.2	72.6	34.7	33.5

The findings described here suggest such an explanation. Analyses designed to identify the relationship between tooth-specific caries susceptibility and the caries-inhibitory effect of fluoride will be given in a subsequent report.

From a practical point of view, the findings direct attention to the pertinent observation that even after prenatal exposure and a postnatal residence of 15-19 years in a fluoride environment, caries attack is reduced only by about a third of normal expectation in the lower molar teeth of lifetime residents. Although this is a significant reduction, particularly from the point of view of the total need for dental service in the population, it is necessary to recognize that approximately two-thirds of the treatment problem for caries still arises among molars in the fluoride area; a phenomenon which probably explains why practicing dentists in that area did not, previous to the present study, independently consider that the teeth of their patients were uniquely different from those of other children in the United States.

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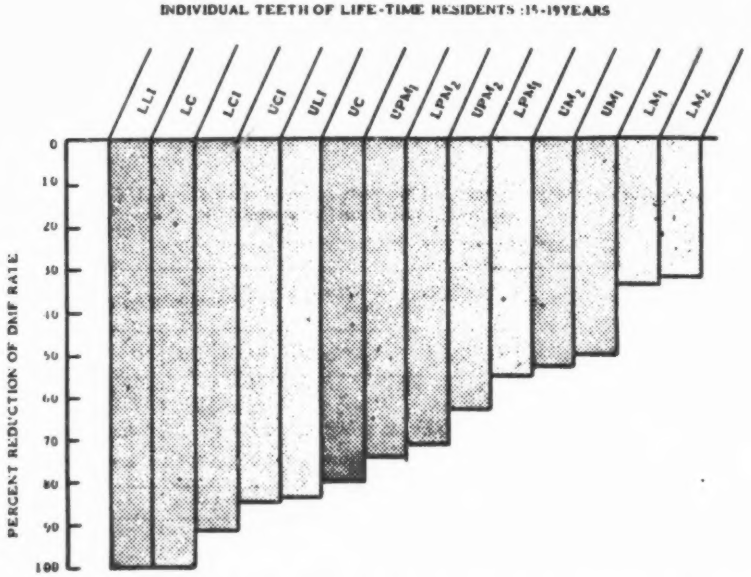

FIGURE 3.—*Percent reduction below corresponding rates for Hagerstown children in number of DMF teeth of specified kinds, left and right sides of the upper and lower jaws (maxilla and mandible) per 100 children of both sexes, 15–19 years of age, lifetime residents of a fluoride area of New Jersey. LLI—lower lateral incisor, LC—lower canine, LCI—lower central incisor, UCI—upper central incisor, ULI—upper lateral incisor, UC—upper canine, UPM—upper first premolar,* LPM_2*—lower second premolar,* UPM_2*—upper second premolar, LPM—lower first premolar,* UM_2*—upper second molar,* UM_1*—upper first molar, LM—lower first molar,* LM_2*—lower second molar.*

TABLE 4.—*Number of migrants and number of DMF teeth per 100 children (both sexes) and the duration of exposure in a fluoride area of New Jersey*

Age (years)	Exposure (years)	Number persons	Maxillary teeth left and right sides							Mandibular teeth left and right sides						
			CI	LI	C	PM_1	PM_2	M_1	M_2	CI	LI	C	PM_1	PM_2	M_1	M_2
5-9	0-4	175	0	0	0	0	0	21.7	0	0	0	0	0	0.6	35.4	0
	5-9	59	0	0	0	0	0	11.9	0	0	0	0	0	0	22.0	0
10-14	0-4	136	14.0	16.9	0	8.8	8.1	123.5	17.6	2.2	.7	.7	1.5	10.3	148.5	41.2
	5-9	118	5.1	4.2	0	4.2	5.9	83.9	11.0	0	0	0	0	4.2	103.4	26.3
	10-14	53	5.7	7.5	0	3.8	5.7	52.8	5.7	0	0	0	7.5	7.5	81.1	24.5
15-19	0-4	45	51.1	48.9	11.1	40.0	51.1	128.9	71.1	2.2	0	0	20.0	55.6	148.9	108.9
	5-9	67	46.3	38.8	9.0	34.3	25.4	147.8	61.2	1.5	1.5	3.0	6.0	28.4	155.2	91.0
	10-14	58	22.4	22.4	0	22.4	29.3	113.8	56.9	3.4	3.4	1.7	10.3	13.8	139.7	77.6
	15-19	14	0	0	0	14.3	7.1	42.9	7.1	0	0	0	0	0	71.4	35.7

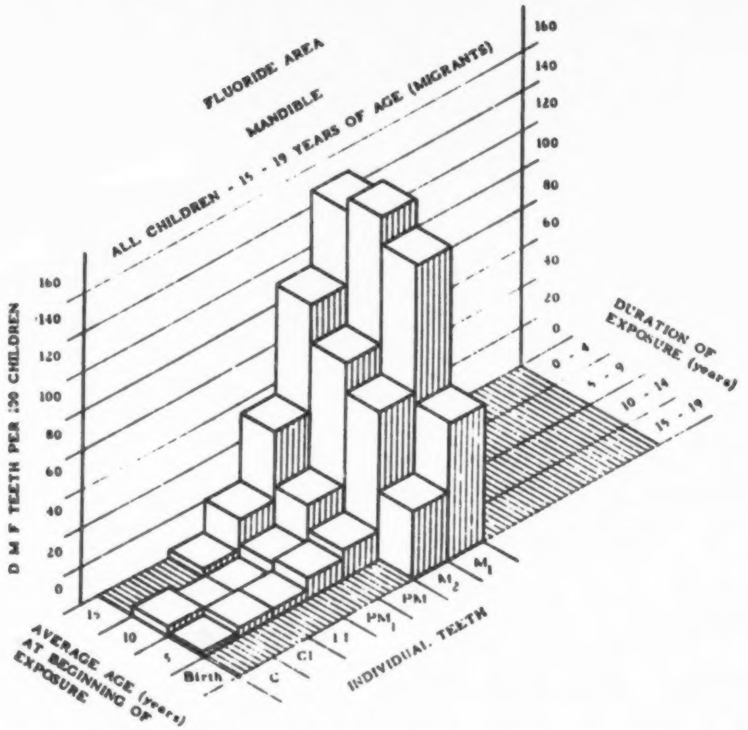

FIGURE 4.—*Number of DMF teeth of specified kinds, left and right sides of the lower jaw (mandible) per 100 children of both sexes, 15–19 years of age; by duration of exposure in a fluoride area of New Jersey. CI—central incisor, LI—lateral incisor, C—canine, PM—first premolar, PM_2—second premolar, M—first molar, M_2—second molar.*

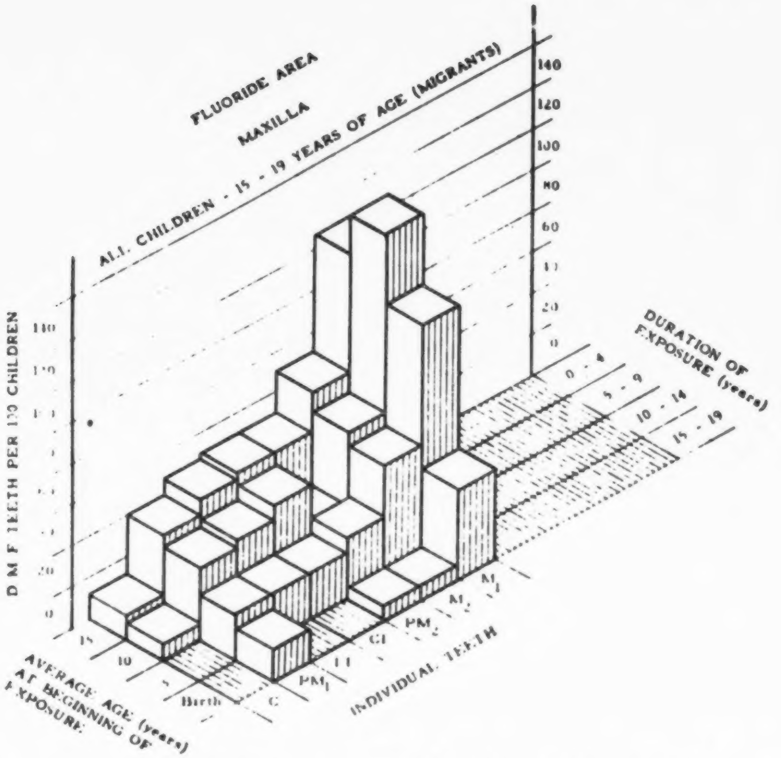

Figure 5.—*Number of DMF teeth of specified kinds, left and right sides of the upper jaw (maxilla) per 100 children of both sexes, 15–19 years of age; by duration of exposure in a fluoride area of New Jersey. CI—central incisor, LI—lateral incisor, C—canine, PM—first premolar,* PM_2*—second premolar, M—first molar,* M_2*—second molar.*

DOMESTIC WATER AND DENTAL CARIES. VII. A Study of the Fluoride-Dental Caries Relationship in an Adult Population

By A. L. Russell and Elias Elvove

Exhaustive studies of the fluoride-dental caries relationship in children have invariably demonstrated a marked inhibition of the disease in children who have always used a domestic water containing 1.0 part per million or more of fluorides. In adults, however, very few such studies have been reported and the findings are not in general agreement. Weaver concluded that fluoride ingestion had merely postponed the onset of dental caries by about 5 years in 100 young British mothers (*1*, *2*). Deatherage, on the contrary, observed caries-inhibitory effects which were still pronounced in Illinois males with a mean age of 25 years (*3*, *4*), while McKay, after a study of natives of Colorado Springs, concluded that "the inhibitory effect of fluoride, once acquired, is permanent" (*5*). Further study of the fluoride-dental caries relationship in representative adult populations seemed to be indicated.

THE STUDY CITIES

Colorado Springs, Colo., was selected for the present investigation, principally because of its long and reliable fluoride history. Nearby Boulder, Colo., was utilized as a control.

The populations of the two cities were 36,789 and 12,985, respectively, in 1940. Both lie in the semiarid plain at the eastern border of the Rocky Mountains, at altitudes of 6,098 and 5,404 feet. Since 1906 their average mean annual temperatures have been 48.1° and 50.6° and their mean annual precipitations, 14.8 and 18.9 inches, respectively; during this period the two weather stations have recorded about the same number of clear, partly cloudy, and cloudy days.

Native-born whites made up 98 percent of the Boulder population and 96 percent of the Colorado Springs population in both 1930 and 1940. Neither can be considered an aging nor a young population with respect to the other; both are highly literate. In general, workers in the two communities followed much the same occupations and earned about the same amounts in wages or salaries. Compared with the United States as a whole, the two communities depend for income rather more upon wholesale and retail trade, the professions, and services of various kinds, and sharply less upon manufacturing.

THE WATER SYSTEMS

The two water systems are essentially similar. In both, water is collected and stored in well-demarcated mountain watersheds and conveyed to reservoirs in or near the cities through closed pipeline systems. The pipelines and the underlying granites of the watersheds preclude the entry of ground water at any point. Except for the enlargement of storage facilities within the present watersheds, neither has been significantly altered since the summer of 1906, when both pipeline systems were put in operation. Colorado Springs, in December of 1950, began to take about 2 million gallons of water per day from Fountain Creek, which drains an area somewhat north of the present watershed. Clinical examinations were completed there during the following week. A sample of Fountain Creek water analyzed by Elvove in October 1944 contained 2.8 parts per million of fluorides.

Colorado Springs Water System

An earlier report described the Colorado Springs water system in detail and summarized the epidemiological evidence that fluorine has been a constituent of Colorado Springs tap water for more than 50 years (*6*). Further evidence that a constant amount of fluoride has been present in Colorado Springs water for at least 44 years will be presented in this report. Twelve consecutive monthly samples of Colorado Springs tap water have twice been analyzed at the National Institute of Health. Between November 1933 and October 1934 the mean annual level was 2.5 p.p.m. of fluorides, with a high content of 3.0 p.p.m. in February, March, and April and a low of 1.8 p.p.m. in August (7). Between March of 1940 and February of 1941 a similar series of analyses showed a mean annual content of 2.55 p.p.m. of fluorides, with a high level of 2.8 p.p.m. in March and a low of 2.4 p.p.m. in June and August (*6*).

The Boulder Water System

The only known fluoride deposit in the Boulder watershed is about 60 feet below the surface and is virtually inaccessible to water in the system itself (*8, 9*). The entire watershed, comprising 19.3 square miles, is limited on the west by the Continental Divide and on the north and south by steep ridges varying in elevation from 11,100 to 13,515 feet. Its rocks are mainly pre-Cambrian schists and gneisses, with intrusions of tertiary granite and pegmatites (*8*). Water for winter use is stored in 11 mountain reservoirs which drain about 8.7 square miles of the watershed proper.

From these reservoirs the water flows for 17,800 feet through a pipeline into the Lakewood reservoir which during the warmer months, drains the entire watershed; thence through 13.6 miles of pipeline to two reservoirs at the edge of the city; and finally into the distribution mains.

Twelve samples of Boulder tap water collected at monthly intervals between March of 1950 and February of 1951 have been analyzed for chemical content by Elvove. These findings are presented in table 1. Only trace amounts of fluoride were found, and these only in the samples collected in April, May, and August of 1950.

A sample of Boulder tap water analyzed by the same chemist in 1945 presented much the same composition.

ASSEMBLY OF EXAMINATION LISTS

The examination lists were based upon school census records, birth records, marriage records, and city directories; the same compilation procedure was followed in both cities. Age limits of 20 through 44 years were established because younger persons were not listed in the directories, and because Boulder tap water may have contained fluorides from adjacent watersheds prior to establishment of the pipe-line system in 1906. A white male was included in the sample if he was listed as a student in the school census record for 1920, 1930, or 1940 and as a resident in the current city directory, and if his birth record indicated that his mother's usual residence at the time of his birth was accessible to the city water system as it then existed. The same procedure was followed for white females, except that it was necessary to procure the married name from the county marriage records before the lists could be checked against the birth records or the directories.

THE STUDY SAMPLES

When assembled, the sample lists were compared for race and occupation with 1940 census data. The samples proved to be random cross sections of all the people of the two communities. An attempt was made to examine each listed person, and it is believed that about five-sixths of the actual number of eligible persons was examined in each community.

All of the persons reported upon here were white native residents of one of the two cities. In each community about 90 percent of their parents had been born in the United States, about 3 percent in the British Empire, about 3 percent in one of the Scandinavian countries, and about 1 percent in Central Europe. More than half of the women were housewives and most of the rest were clerical and

Table 1.—*Analyses of water samples from Boulder, Colo.*

Composition	Mar. 1950	Apr. 1950	May 1950	June 1950	July 1950	Aug. 1950	Sept. 1950	Nov. 1950	Dec. 1950	Jan. 1951	Feb. 1951	Mar. 1951
	Parts per million											
Total dissolved solids (103° C.)	21.9	32.0	27.2	18.8	15.2	16.0	16.0	26.4	24.0	16.0	32.0	36.0
Loss on ignition	3.5	10.8	10.0	4.4	3.6	4.8	4.0	5.6	4.8	4.0	8.0	8.8
Fixed residue	18.4	21.2	17.2	14.4	11.6	11.2	12.0	20.8	19.2	12.0	24.0	27.2
Silica (SiO_2)	4.0	2.4	6.4	4.0	1.6	1.6	2.4	4.0	4.0	6.4	9.6	10.4
Iron (Fe)	.08	.15	.22	.08	.03	.08	.14	.14	.12	.02	.10	.08
Aluminum (Al)	.02	0	.05	.01	0	0	.01	.01	.01	0	0	.02
Calcium (Ca)	4.6	4.6	4.6	2.3	2.3	2.3	2.3	4.0	3.4	1.7	4.0	4.0
Magnesium (Mg)	.9	1.4	.9	.5	.7	.7	.7	.9	.7	.5	1.2	1.2
Sodium and potassium (calculated as Na)	.8	2.0	.5	2.2	1.2	.2	.2	.7	.3	.2	.2	.9
Carbonate (CO_3)	0	0	0	0	0	0	0	0	0	0	0	0
Bicarbonate (HCO_3)	19.5	20.7	15.9	12.2	12.2	9.8	9.8	12.2	12.2	4.9	17.1	17.1
Sulfate (SO_4)	2.9	1.2	2.1	2.2	2.5	1.6	2.5	1.2	3.3	2.5	3.3	2.9
Nitrate (NO_3)	.6	.3	.4	.4	.3	.2	.3	.2	.2	.2	.3	.2
Chloride (Cl)	.1	.1	.2	.1	.1	.1	.1	.1	.1	.1	.1	.2
Phosphate (PO_4)	0	0	0	0	0	0	0	0	0	0	0	0
Fluoride (F)	0	.1	.1	0	0	.1	0	0	0	0	0	0

TABLE 2.—*Individual fluorosis ratings of Colorado Springs natives, 1950*

Age group	Number of persons	Numbers with stated degree of fluorosis				
		Normal or questionable	Very mild	Mild	Moderate	Severe
20–24	72	15	29	20	7	1
25–29	101	18	41	35	6	1
30–34	82	15	41	16	8	2
35–39	75	10	34	24	6	1
40–44	55	5	29	14	5	2
Total	385	63	174	109	32	7

sales workers. Most of the men were professional or semiprofessional workers, business proprietors, skilled craftsmen, or students. There were comparatively few unskilled or semiskilled workers in either group. As estimated from 1940 census data, the past per capita incomes of the two groups seem to have been very nearly the same. College graduates made up a high percentage of each sample—about 40 percent at Boulder and about 20 percent at Colorado Springs. Both groups had received adequate dental care of high quality and personal dental hygiene was generally good.

CRITERIA AND METHOD OF EXAMINATION

Clinical dental examinations were conducted in Boulder in July and August 1950, and in Colorado Springs during September, October, and December of the same year. All examinations were made by the senior author, using mouth mirror and explorer. Most of the examinations were made by appointment at a central clinic where a dental chair and operating light were available. Nonresponse was tested through house calls or calls at the place of employment; examinations away from the clinic were made under light from a Hood head lamp.

"Catches," or deep pits and fissures, were not considered carious in the absence of other indications of caries. The outline of each lesion or restoration was sketched on a morphological tooth chart by the examiner. The primary reason for extraction, based upon a history of signs and symptoms, was recorded for each missing tooth. The condition of the gingival tissues was assessed in detail. The criteria for fluorosis followed those established by Dean (*10*). Also recorded for each examinee were his name, age, sex, present address, usual occupation, number of school years completed, water and residence histories, and the salient facts of his health history. The examination and history taking required about 15 minutes per person.

The findings for each examinee were included in the tabulation if his residence and usage of the local water were unbroken except for periods not exceeding 60 days during the commonly accepted calcification and eruption periods of the permanent teeth, and if thereafter he had spent more than half of his life in residence with use of the water in question. The commonest history showed unbroken residence for 18 to 20 years, departure to enter school or the armed forces, and return when schooling or the period of service had been completed.

In tabulation a tooth with an unfilled carious lesion plus a restoration was counted as a filled tooth.

FINDINGS

FLUOROSIS

All of the eligible persons examined at Boulder were free of fluorosis.

The distribution of fluorosis ratings by individuals at Colorado Springs is shown in table 2. The odds are about five to one that all variations from the common pattern are due to chance. This finding supports the deduction of Dean, Arnold, and Elvove that "the inhabitants of Colorado Springs . . . have been using a relatively similar type of water for at least as long as 60 years" (*6*).

DECAYED, MISSING, OR FILLED PERMANENT TEETH

The age-sex composition of the study groups, with their rates for DMF (decayed, missing, or filled permanent) teeth are summarized in table 3 and pictured in figure 1. For purposes of comparison, DMF rates reported for insurance company employees in New York City by Hollander and Dunning (*11*) are also shown in figure 1. The Boulder rates lag the New York rates at the three younger age points. Delayed onset of dental caries may be typical of this geographical area. Senn reported a DMF rate of 11.8 for Colorado aviation cadets against a rate of 19.4 for similar cadets from the State of New York (*12*). While his Colorado group probably included men from fluoride as well as nonfluoride areas, his rates for Montana and Wyoming are also low (10.5 and 13.1, respectively) and fluoride waters are less common in the two latter States. Schlack and Birren found an average of 11.7 dental defects per man in 3,672 naval recruits from Arizona, Colorado, Idaho, Montana, Nevada, Utah, and Wyoming, compared with 17.6 per man in 12,972 recruits from New Jersey, New York, and Pennsylvania (*13*). Particularly at Boulder there was a tight grouping of individual findings around the average finding so that standard deviations are narrow in ratio to the rates, and the standard errors are small. Hence, these rates are more dependable than rates taken from typical samples of children many times larger would have been (*14*). They plot smoothly and follow the general shape of the Colorado Springs curve except at ages 40-44, where the standard error is wide and the actual finding may be somewhat low. The differences between the two communities are statistically valid at each age point and for the pattern as a whole.

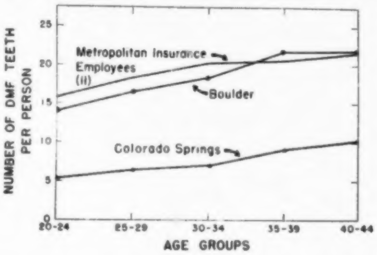

FIGURE 1.—*Total DMF rates, third molars excluded, in adult natives of Boulder and of Colorado Springs, Colo., compared with rates reported for Metropolitan Life Insurance Company employees.*

In children the DMF rate is a measure of dental caries experience and little else. In adults it also includes an accumulation of teeth lost from periodontal disease or from trauma, or restored because of fracture, erosion, and the like. Those fractions of the present rates which were due primarily to causes other than dental caries are indicated in table 3. They are approximately equal in the two study groups—amounting, overall, to about 1.0 tooth per person at Boulder and to about 0.8 tooth per person at Colorado Springs. Hence the disparity in the rates is due to a difference in dental caries experience in the two populations. The Colorado Springs rates is about 60 percent less than the Boulder rate at each age point. This is about the same magnitude of dental caries inhibition previously reported for children who were natives of a fluoride area (*15*).

Filled Tooth Surfaces

In each group about three-quarters of all DMF teeth were filled. There was a tendency toward pit-and-fissure fillings at Colorado Springs, and toward multisurface restorations at Boulder. This difference is conservatively expressed by the ratio of filled surfaces to filled teeth. At all ages the mean number of filled surfaces per filled tooth was slightly more than two at Boulder and slightly more than one and one-half at Colorado Springs. The ratios for all teeth, by age groups, are shown in figure 2.

Caries-Free Individuals

No person in the Boulder group was caries-free. Caries-free individuals made up the following percentages of persons examined at Colorado Springs:

Age-group	*Colorado Springs, percent caries-free*
20–24	26.4
25–29	7.9
30–34	7.3
35–39	9.3
40–44	1.8

Tooth Mortality

Data concerning tooth mortality in the two communities are given in table 4. The rates are significantly different at each age point and for the pattern as a whole. Compared with Colorado Springs, Boulder natives had lost more than three times as many teeth from dental caries, appreciably but not significantly more from periodontal disease, and slightly and insignificantly fewer from other causes.

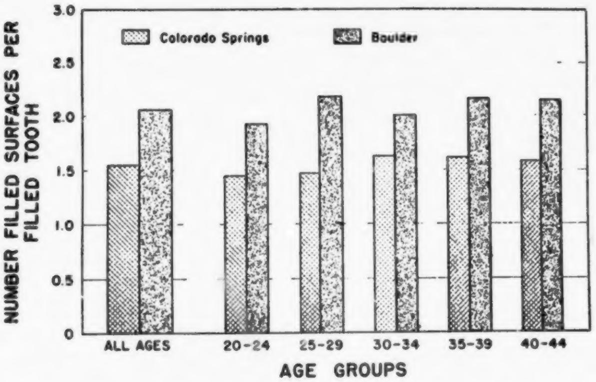

Figure 2.—*Mean numbers of filled tooth surfaces per filled tooth in adult natives of Boulder and of Colorado Springs, Colo.*

When tooth mortality rates are plotted against the number of school years completed per person (fig. 3), there is a considerable difference in favor of those persons who had completed one or more years of college. This is not due to a preponderance of college people at the younger ages and of noncollege people at the older ages, a fact illustrated by the age-specific breakdown in figure 4.

Since two-thirds of the Boulder group were college people and two-thirds of the Colorado Springs group were noncollege people, it is clear that tooth mortality rates must be adjusted for this factor if direct comparisons between the two groups are to be considered valid. Tooth mortality rates so adjusted are listed in table 5 and portrayed in figure 5. The effect of this adjustment is to raise Boulder tooth mortality rates as compared with Colorado Springs. On this basis Boulder natives have lost about four times as many teeth from dental caries as have natives of Colorado Springs.

Table 3.—*Total numbers of decayed, missing, and filled permanent teeth in adult natives of Boulder and Colorado Springs, Colo., excluding third molars*

Age group, years	Number of persons			Numbers of teeth				DMF rate	Standard deviation of the rate	Standard error of the rate	Segment of rate due to	
	Male	Female	Both	Decayed	Missing	Filled	Total DMF				Periodontal disease	Other causes
						Boulder						
20–24	22	29	51	56	64	596	716	14.0	4.9	0.69	....	0.2
25–29	26	15	41	49	104	523	676	16.5	5.5	.86	....	.5
30–34	17	12	29	33	116	381	530	18.3	5.2	.97	1.9	.1
35–39	8	14	22	14	250	216	480	21.8	5.1	1.09	1.3	.1
40–44	6	6	12	11	145	104	260	21.7	6.0	1.73	2.3	.3
All ages	79	76	155	163	679	1,820	2,662	17.2	....	....	.7	.3
						Colorado Springs						
20–24	36	36	72	16	41	332	389	5.4	5.1	0.60	....	0.2
25–29	61	40	101	30	90	536	656	6.5	5.0	.50	....	.3
30–34	55	27	82	10	93	476	579	7.1	4.9	.54	....	.3
35–39	51	24	75	13	240	435	688	9.2	7.0	.81	1.0	.6
40–44	36	19	55	7	170	388	565	10.3	6.4	.86	.6	1.0
All ages	239	146	385	76	634	2,167	2,877	7.5	....	....	.3	.5

Third Molars

Third molars have been excluded in all of the data so far presented.

In both populations the percentages of third molars in eruption which were decayed, missing, or filled was high, rising with age from 70 to nearly 100 percent at Boulder and from 50 to over 90 percent at Colorado Springs. About three-quarters of all DMF third molars were missing in both groups. At Boulder 94 percent of third molar loss was reported as due to dental caries and about three percent as due to malposition of the teeth. At Colorado Springs about 36 percent of third molar loss was reportedly due to dental caries and about 62 percent to malposition of the teeth.

DISCUSSION

The most reliable measure of the difference in the dental caries experience of these two groups is the difference between their total DMF rates (table 3 and fig. 1). These rates were not disturbed by differences in sex, education, or economic status, and so far as they are concerned the two groups seem to be directly comparable in every respect except use of a fluoride-free water on the one hand and use of a fluoride-bearing water on the other. They support the findings of Deatherage (*3, 4*) in all re-

Table 4.—*Tooth mortality experience in adult natives of Boulder and of Colorado Springs, Colo., third molars excluded*

Age group, years	Number persons	Number missing teeth	Total tooth mortality rates			Teeth missing due to—					
			Mortality rate	Standard deviation	Standard error	Dental caries		Periodontal disease		Other	
						Number	Rate	Number	Rate	Number	Rate
Boulder											
20–24	51	64	1.26	2.10	0.29	53	1.04	-------	-------	11	0.22
25–29	41	104	2.54	3.71	.58	88	2.15	1	0.02	15	.37
30–34	29	116	4.00	7.16	1.33	59	2.03	56	1.93	1	.03
35–39	22	250	11.36	11.94	2.55	221	10.05	28	1.27	1	.05
40–44	12	145	12.08	11.37	3.28	116	9.67	27	2.25	2	.17
All ages	155	679	4.38	-------	-------	537	3.47	112	.72	30	.19
Colorado Springs											
20–24	72	41	0.57	1.50	0.18	28	0.39	2	0.03	11	0.15
25–29	101	90	.89	1.54	.15	74	.73	1	.01	15	.15
30–34	82	93	1.13	1.93	.21	75	.92	1	.01	17	.21
35–39	75	240	3.20	6.42	.74	122	1.63	77	1.03	41	.55
40–44	55	170	3.09	5.55	.75	100	1.82	32	.58	38	.69
All ages	385	634	1.65	-------	-------	399	1.04	113	.29	122	.32

spects and, although somewhat higher than those reported from a less representative group, are in accord with the principal conclusions of McKay (*5*). Since this report was prepared for publication a personal communication describing a comparable study has been received from Peter Adler. Adler found that female natives of Kunszemarton, Hungary, where the water contains from 0.8 to 1.6 p.p.m. of fluorides, showed lower dental caries rates through the age range of 21 to 45 years than did newcomers to Kunszemarton or comparable natives of Nagybaracska, Debrecen, and vicinity who had used waters which were low in fluoride.

Direct comparisons with Weaver's data are not valid since he presents essentially tooth mortality rates; in his nonfluoride and fluoride groups 86.2 percent and 82.4 percent, respectively, of all DMF teeth were missing or indicated for extraction. His sample was highly selected—"mothers attending maternity and child welfare centers," of whom the great majority "had not well-cared-for mouths" (*2*). The present findings (fig. 4) indicate that tooth mortality may be affected by factors other than the severity of dental caries per se. For example, a considerable tooth loss from periodontal disease may occur in Colorado citizens with less than average schooling at the ages considered by Weaver in his study. None of the data for the Colorado groups suggest that Colorado Springs caries rates will "catch up" with those for Boulder natives inside a normal human life span.

SUMMARY AND CONCLUSIONS

1. Objective lists of natives 20–44 years old with verified histories of residence and water usage were prepared in Boulder, Colo., where the communal water is virtually fluoride-free, and in Colorado Springs, Colo., where the communal water contains about 2.5 p.p.m. of fluorides. The cities are otherwise similar and the two groups are comparable in race and occupation. Persons on the lists were given dental examinations with mouth mirror and explorer.

2. The prevalence of fluorosis was uniform through the age range at Colorado Springs. No fluorosis was seen in natives of Boulder.

3. Total rates for decayed, missing, or filled permanent teeth were about 60 percent lower in Colorado Springs than in Boulder for each age group. The phenomenon of caries inhibition continued undiminished through the age of 44 years.

4. Boulder natives had lost three or four times as many teeth from dental caries as had natives of Colorado Springs.

5. The observed caries-inhibitory effect was essentially similar in pattern and in magnitude to that seen in children native to fluoride areas.

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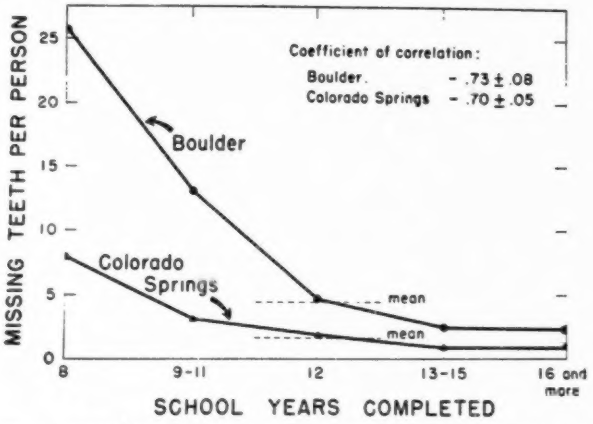

Figure 3.—*Tooth mortality rates in the two groups of Boulder and of Colorado Springs, Colo., natives, by number of school years completed.*

TABLE 5.—*Tooth mortality rates in natives of Boulder and of Colorado Springs, Colo., adjusted for numbers of school years completed*

Age group	Total mortality rate	Fraction of rate due to		
		Caries	Periodontal disease	Other
Boulder				
20-24	1.38	1.19	----------	0.19
25-29	3.27	2.83	0.05	.39
30-34	4.25	2.04	2.17	.04
35-39	11.41	10.08	1.28	.05
40-44	14.68	11.09	3.40	.19
All ages	5.33	4.07	1.06	.19
Colorado Springs				
20-24	0.48	0.33	0.02	0.13
25-29	.84	.69	.01	.14
30-34	1.09	.86	.01	.22
35-39	3.10	1.61	.96	.52
40-44	2.94	1.76	.54	.65
All ages	1.55	.99	.27	.30

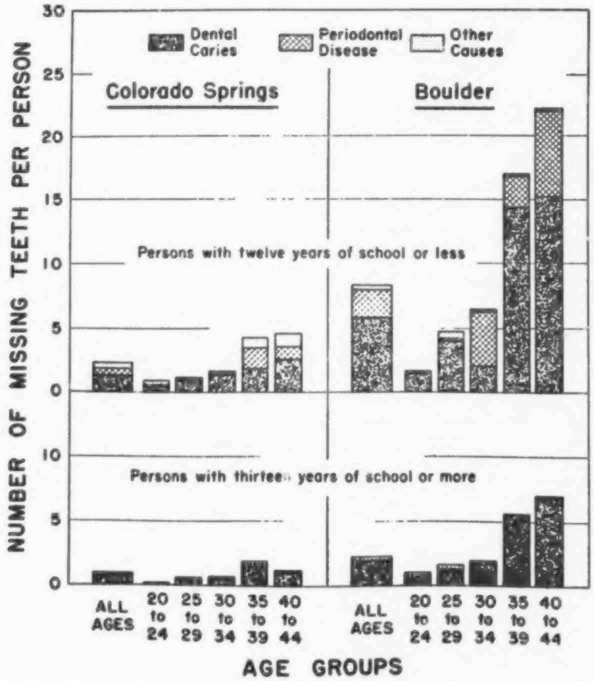

FIGURE 4.—*Tooth mortality rates in Boulder and in Colorado Springs, Colo., natives with 12 years of school or less and with 13 years of school or more, third molars omitted.*

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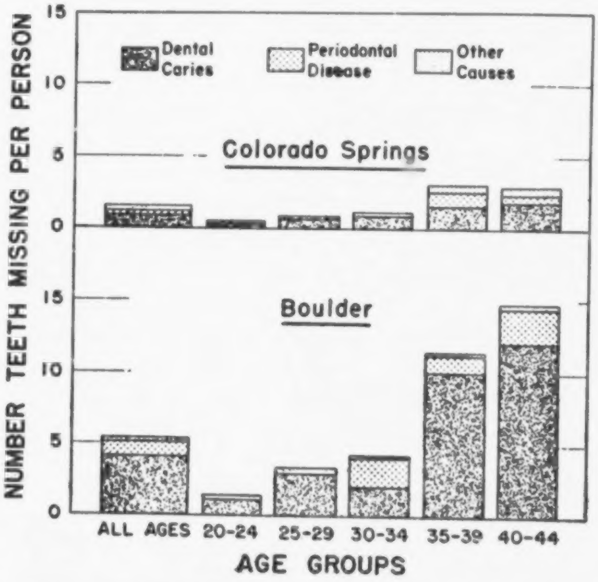

FIGURE 5.—*Tooth mortality rates in natives of Boulder and of Colorado Springs, Colo., adjusted for the common number of school years completed.*

MISSOURI'S FLUORIDE AND DENTAL CARIES STUDY

GEORGE A. NEVITT, VIRON DIEFENBACH, and CHARLES E. PRESNELL

A Study of the Dental Caries Experience and the Fluoride Content of the Drinking Water of 3,206 White Children in Nine Selected Cities in Missouri

Numerous studies of the fluoride-dental caries relationship have been made. An inhibition of dental caries disease has been reported in children who have regularly used a domestic water supply containing approximately 1.0 part per million of fluorides naturally present in their water supply (*1, 2, 3, 4*). No adverse effects from the 1.0 part per million of fluorides in a public water supply have been noted (*5, 6*). The use of fluoridated water gives the same beneficial effects as does the use of natural fluoride water of a similar concentration (*7, 8, 9, 10, 11*).

STUDY

The study described here was made for the purpose of obtaining data of the dental caries experience in continuous resident white children, ages 6 through 14, living in nine selected Missouri cities with public water supplies bearing different fluoride concentrations naturally. This was a cooperative study made by the Missouri Division of Health and the Public Health Service Regional Office VII.

THE STUDY OF CITIES, THEIR CLIMATES AND POPULATIONS

Nine cities, which are located in a geographic band stretching across Missouri from St. Louis to Kansas City, were selected (fig. 1) Generally, the cities lie in the 37°–39° parallels of latitude area. The climatological data is similar for all nine study cities. There are only 2° difference in the mean annual temperatures between the most northern and southern cities, and the precipitation, growing season and humidity are very nearly the same for all of them (*12, 13, 14, 15*). (Appendix table 1).

The population of the study cities is made up largely of people who migrated from the eastern border states of Missouri, i.e., Kentucky, Illinois, and Tennessee (*16*). Census statistics show that 99 per cent of all white residents in these communities are native born (*17, 18*).

The industry of this area of Missouri is predominantly agriculture. The economic pattern is one of the mutual dependence between the urban centers and the surrounding rural areas. Generally the rural level of living is a guide to the entire area, and Hagood has rated this level as average when compared to the national level of living (*19*). In addition to agriculture, the communities in this study have other industries which tend to stabilize the economy in periods of poor farm yield.

SELECTION OF STUDY GROUPS

The purpose and methods of the study were explained to the local dentists, health units, school authorities and school nurses in each city. Arrangements were made for the resident history questionnaires to be filled out by the entire white school census between the ages of 6 and 14 years.

All of the children who qualified in each city were included in the study. The sample was composed of: (*1*) White children between the ages of 6 and 14 years inclusive; (*2*) Children who had a verified continuity of residence from birth in their city; and (*3*) Children who had used the public water supply as their regular source of water.

The selection of children for the dental examination was made in two steps.

Selection Step No. 1—The completed questionnaires were returned to the examiners for review. The names of the children whose answers indicated that they were continuous residents from birth and had used the public water supply as their regular source of water were placed on the dental examination list.

Selection Step No. 2—Before the dental examination was made, each child on the

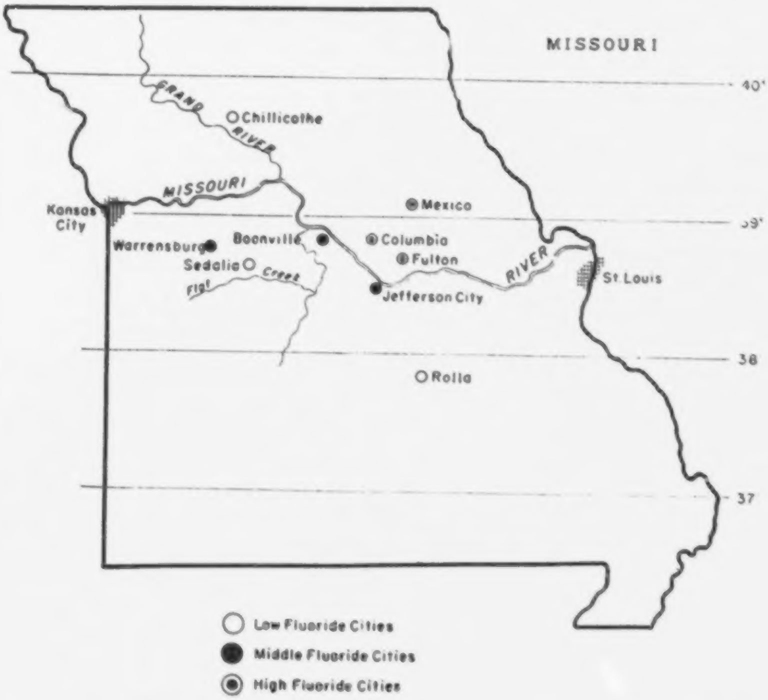

FIGURE 1.—*Diagrammatic map of Missouri indicating the location of the selected cities in the Missouri dental study and the parallels of latitude.*

examination list was interviewed by a member of the survey team. At this interview the child's source of water supply and his continuity of residence were verified. Children who had received topical fluoride treatments were not included in the study. The school nurse was present to add her personal knowledge of the history of each child.

CRITERIA OF DENTAL CARIES AND METHOD OF EXAMINATION

A tooth was considered to be carious if the lesion was clinically obvious, if the opacity of the enamel indicated underlying caries, or if either end of a No. 5 double-end dental explorer could be made to penetrate into soft yielding tooth structure. Pits or fissures which did not present one of the above criteria were not considered to be carious even though the dental probe could be made to catch.

Permanent Teeth.—Permanent teeth were classified in regard to dental caries experience. Each tooth with positive caries experience was classified as either *decayed, missing,* or *filled* and scored as one against the patient. The total score for each patient was the sum of the decayed, missing, and filled teeth. No tooth was scored more than once. A tooth which was both carious and filled was recorded as carious. Teeth which were missing or restored as a result of a reported traumatic injury were not recorded as DMF teeth. Teeth extracted for orthodontic reasons or congentially missing were not counted. Third molar teeth are rarely found in children of these ages and therefore were not counted.

Deciduous Teeth.—Classification of dental caries in the deciduous teeth was made with respect to the entire dentition rather than individual teeth. The entire deciduous dentition was recorded as a plus, a minus, or a zero. A plus indicated the presence of one or more decayed or filled deciduous teeth; a minus indicated no caries experience in the deciduous teeth present; and a zero indicated that no deciduous teeth were present. Since the exfoliation time for deciduous teeth varies considerably among individuals, it is of doubtful value to compare missing tooth rates in the primary dentition of older age groups.

Dental Examination Procedure.—Uniformity of diagnoses of the two examiners was established before the study was begun and maintained throughout the study by a standardization technique. This was done by the operators independently examining the same child and then comparing diagnoses. A small group of children was used for this purpose in each study city.

No advance notice was given to the children or parents about the dates of the examinations. The examinations were done alphabetically by age groups and children who were not in attendance on the day of the examinations were dropped from the study. The clinical findings were dictated to a recorder.

THE WATER SYSTEMS

Although these cities are close neighbors, the fluoride concentrations naturally present in their water supplies are different. A review of the fluoride tests made on these water supplies between 1935 and 1951 shows that the mean fluoride determinations range between 0.08 part per million and 1.32 parts per million (table 1). The nine cities were divided into three groups according to the fluoride content of their water supplies. On this basis they were classified as low fluoride cities (0.1 to 0.2 p.p.m.), middle fluoride cities (0.4 to 0.6 p.p.m.), and high fluoride cities (0.9 to 1.2 p.p.m.). Due to differences among the study cities in the number of children examined, the mean fluoride determination for each group of cities has been adjusted according to the size of the sample The results show that the three low fluoride cities have an adjusted mean fluoride determination of 0.2 part per million; the three middle fluoride cities have an adjusted mean fluoride determination of 0.5 part per million; and the three high fluoride cities have an adjusted mean fluoride determination of 1.1 parts per million.

TABLE 1.—*Summary of the fluoride determinations made on the water supplies between 1935 and 1952 of the selected cities in the Missouri dental study*

City	Range of F. Determinations 1935–1952 p.p.m.	Annual mean F. Determinations 1935–1952 p.p.m.	Median F. Determinations 1935–1952 p.p.m.	Adjusted mean F. Determinations[1] 1935–1952 p.p.m.
Rolla	0.05–0.10	0.08	0.1	0.2
Sedalia	0.05–0.25	0.18	0.2	
Chillicothe	0.05–0.45	0.26	0.2	
Boonville	0.05–0.55	0.42	0.4	0.5
Jefferson City	0.25–0.60	0.49	0.5	
Warrensburg	0.50–1.30	0.68	0.6	
Fulton	0.70–1.00	0.87	0.9	1.1
Mexico	0.10–1.50	0.87	1.0	
Columbia	0.90–2.20	1.32	1.2	

[1] On the basis of the relative numbers of children examined.

The records of the fluoride determinations were made on the water supplies of the study cities from 1935 to 1952—the years during which the teeth of the sample children were developing—are given in the appendix table 2. The 1940 fluoride determinations of Warrensburg, Mexico, and Columbia; the 1938 determination of Mexico; and the 1942 determination of Columbia were unusually different from their averages of the 1935 to 1952 period. The information available does not offer an explanation of this difference. All of the recorded determinations were made by procedures given in the *Standard Methods for the Examination of Water and Sewage* (8th and 9th Editions). Monthly fluoride tests were made on the water supplies of the study cities from October, 1951, to October, 1952. Generally the fluoride tests made during the period of the study support the fluoride data for the years 1935-1951. The sum total of this fluoride data gives the best approximations that can be made of the fluoride levels prevailing in the water supplies of the study cities.

Information about the source, treatment and chemical analyses of the various water supplies is given in the appendix tables 3 and 4.

Of the nine study cities, five of them depend on deep wells and four of them depend on streams as their source of domestic water supply. The mineral composition of surface water supplies probably fluctuates with seasonal rainfall and runoff whereas the composition of deep well water ordinarily shows little fluctuation. Twelve consecutive monthly chemical analyses would be desirable, especially of the surface water supplies. The analyses given should not be taken literally as representing the average mineral composition of the water supplies used throughout the year but only as indications of the general chemical composition.

FINDINGS

DISTRIBUTION OF THE STUDY SAMPLE

The distribution of the sample was as follows: 1,224 children in the low fluoride cities (0.2 p.p.m.); 950 children in the middle fluoride cities (0.5 p.p.m.); and 1,032 children in the high fluoride cities (1.1 p.p.m.). The distribution according to sex was almost evenly divided in all

Table 2.—*Summary of the distribution of the 3,206 children examined, according to age and sex, and classified by cities having low, middle and high natural fluoride bearing water supplies*

City	Number of children examined			Summary of the distribution of the 3,206 children examined, according to age, and classified by cities having low, middle and high natural fluoride bearing water supplies								
				Age in years last birthday								
	Male	Female	Total	6	7	8	9	10	11	12	13	14
				Number of children								
Low Fluoride Cities Avg. F. 0.2 p.p.m.[1]												
Rolla	60	82	142	13	17	21	22	16	15	11	19	8
Sedalia	421	399	820	120	96	122	89	88	78	82	76	69
Chillicothe	139	123	262	39	31	57	30	38	21	17	19	10
Totals	620	604	1224	172	144	200	141	142	114	110	114	87
Middle Fluoride Cities Avg. F. 0.5 p.p.m.[1]												
Boonville	64	58	122	15	16	16	15	18	11	9	14	8
Jefferson City	329	342	671	79	100	80	104	79	73	82	51	23
Warrensburg	71	86	157	14	24	20	26	18	20	16	10	9
Totals	464	486	950	108	140	116	145	115	104	107	75	40
High Fluoride Cities Avg. F. 1.1 p.p.m.[1]												
Fulton	102	100	202	32	35	29	19	20	17	17	16	17
Mexico	149	162	311	43	41	49	33	35	37	35	32	6
Columbia	258	261	519	73	64	66	72	53	60	49	41	41
Totals	509	523	1032	148	140	144	124	108	114	101	89	64

[1] Adjusted for the relative numbers of children examined.

of the cities. An uneven distribution of sex in a sample could influence the data because girls have a higher incidence of caries for each chronologic age than boys (*22*). (Table 2.) There were more younger than older children in the sample because of the continuous resident requirement, but the numbers examined in all age groups were large enough to be significant. There was one large community in each of the low, middle, and high fluoride groups which provided good numbers of children of all ages. The distribution of the sample according to age and sex for each study city is given in the appendix table 5. All eligible children were included in the study except those who were absent on the day of the examination.

Dental Caries Experience

In the data obtained an inverse relationship was found between the total dental caries experience rates and the average fluoride determinations of the low, middle and high fluoride groups of cities (table 3). This finding supports previous studies of the fluoride-dental caries relationship.

The total rates for decayed, missing and filled permanent teeth among the low, middle, and high fluoride groups were markedly different. In the low fluoride group, the sample children experienced an average of 0.91 DMF teeth at 6 years of age increasing to 9.40 at 14 years of age. In the middle fluoride group, the caries experience averaged 0.44 DMF teeth in the 6 year olds and increased to 5.93 in the 14 year olds. The high fluoride group experienced an average of 0.30 DMF teeth at 6 years of age increasing to 1.98 at 14 years of age.

Similarly there was a marked difference in the number of teeth lost by the low and high fluoride cities. In the low fluoride group, the average number of missing teeth ranged from .01 teeth in the 6 year olds to 1.16 in the 14 year olds, while in the high fluoride group the range of missing teeth was from 0.0 in the 6 year olds to only 0.2 in the 14 year olds. The dental caries experience rates are given by age specific groups for each city in the appendix table 6.

Dental Caries Prevalence

Marked differences in the percentages of children affected by the disease with respect to their deciduous and permanent teeth is of interest (table 4). In the low fluoride group 86 to 94 percent of the 6 to 9 year old children were affected by dental caries in their *deciduous teeth* while 60 to 66 percent of the same age

Table 3.—*Summary of the dental caries experience in the permanent teeth of 3,206 white children age 6–14 years, classified by cities having low, middle and high natural fluoride bearing water supplies*

Classification of cities	Avg. F. concentration in p.p.m.	Number of children examined	Classification of dental caries experience	Summary of the dental caries experience in the permanent teeth of the children according to age, classified by cities having low, middle and high natural fluoride-bearing water supplies								
				Age in years last birthday								
				6	7	8	9	10	11	12	13	14
				Average number of permanent teeth affected per child[1]								
The low fluoride cities: Rolla, Sedalia, Chillicothe.	0.2	1,224	Decayed	0.85	1.52	1.81	1.93	2.32	2.87	3.82	5.34	4.31
			Missing	.01	.08	.14	.43	.57	.56	.80	1.17	1.16
			Filled	.05	.33	.87	1.16	1.36	1.82	2.30	2.54	3.93
			DMF	.91	1.93	2.82	3.52	4.25	5.25	6.92	9.05	9.40
The middle fluoride cities: Boonville, Jefferson City, Warrensburg.	0.5	950	Decayed	.41	.68	1.16	.93	1.09	1.66	1.49	1.19	2.60
			Missing			.09	.12	.17	.21	.17	.21	.18
			Filled	.03	.23	.60	1.01	1.43	1.33	2.33	3.15	3.15
			DMF	.44	.96	1.85	2.06	2.69	3.20	3.99	4.55	5.93
The high fluoride cities: Fulton, Mexico, Columbia.	1.1	1,032	Decayed	.25	.40	.34	.55	.48	.39	.54	.85	1.01
			Missing			.01	.06	.09	.04	.11	.29	.20
			Filled	.05	.11	.35	.51	.89	.89	1.22	1.17	.77
			DMF	.30	.51	.70	1.12	1.46	1.32	1.87	2.31	1.98

[1] Adjusted for the relative numbers of children examined.

Table 4.—*Summary of the prevalence of dental caries experience by percent in the deciduous and permanent dentitions of 3,206 white children age 6–14 years, classified by cities having low, middle and high natural fluoride-bearing water supplies*

Classification of cities	Avg. F. concentration in p.p.m.	Number of children examined	Dentition	Summary of the prevalence of dental caries experience in the deciduous and permanent dentitions of 3,206 children according to age, classified by cities having low, middle and high natural fluoride-bearing water supplies								
				Age in years last birthday								
				6	7	8	9	10	11	12	13	14
				Percent of children with dental caries experience[1]								
The low fluoride cities.	0.2	657	Deciduous	87.2	88.9	93.5	85.8					
		1,224	Permanent	41.3	73.6	85.0	90.8	90.1	90.4	95.5	97.4	97.7
The middle fluoride cities.	0.5	509	Deciduous	71.3	80.0	86.2	80.7					
		950	Permanent	23.1	44.3	69.8	71.0	79.1	78.8	83.2	88.0	92.5
The high fluoride cities.	1.1	556	Deciduous	60.1	62.1	63.2	66.1					
		1,032	Permanent	17.6	27.1	34.7	47.6	51.9	52.6	59.4	64.0	62.5

[1] Adjusted for the relative numbers of children examined.

The prevalence of deciduous dental caries experience is confined to children age 6–9 years because of the normal exfoliation of deciduous teeth.

children in the high fluoride group were affected in that respect. Comparing the percentages of children affected by dental caries in their permanent teeth, the low fluoride group ranged from 41.3 percent of the 6 year olds to 97.7 percent of the 14 year olds affected; while in the high fluoride group only 17.6 percent of the 6 year olds to 62.5 percent of the 14 year olds were affected. In the appendix tables 7a and 7b, the percentages of children affected by age specific groups for all cities are given for the deciduous and permanent teeth respectively.

Fluorosis

Almost no evidence of dental fluorosis was observed in Rolla, Sedalia, Chillicothe, Boonville and Jefferson City and only a low percentage of children with mildest forms of dental fluorosis was found in Warrensburg, Fulton, Mexico and Columbia. The clinical evidences of dental fluorosis were all of the mildest forms and of no esthetic significance.

DISCUSSION

Marked differences in the prevalence and amount of dental caries have been demonstrated between 6–14 year old white children of nine Missouri cities having low (0.2 p.p.m.), middle (0.5 p.p.m.), and high (1.1 p.p.m.) fluoride bearing water supplies. It seems unlikely that the differences found in dental caries among the 3,206 children would be due to differences in heredity or diet in these communities. Considering the relatively close geographic locations of these nine cities, the similarity of their climates, the homogeneity of their populations and the comparability of the diagnoses made, it seems very probable that the fluoride present in the water supplies is the dental caries inhibiting factor.

High dental caries experience rates were found associated with the use of domestic waters low in fluorides (0.2 p.p.m.). Low dental caries experience rates were found associated with the use of water supplies high in fluorides (1.1). Intermediately (0.5 p.p.m.) the caries experience rates were lower than those found with relatively fluoride-free waters, but not as low as those found at about 1.0 part per million. The differences in caries experience rates among the fluoride groups of children are significant for each age group throughout the sample.

SUMMARY

1. A dental study of nine Missouri cities with domestic water supplies having different amounts of fluorides naturally present disclosed marked differences in the prevalence and total amount of dental caries experience. An inverse relationship between the dental caries experience and the fluoride concentrations of the water supplies was found.

2. Considering the relatively close geographic locations of the study cities, the similarity of their climates, characteristics of their populations and the sampling method used, it is probable that the observed differences were due to the fluoride factor in their water supplies.

3. There were 3 to 4 times more permanent teeth attacked by caries in the low fluoride cities (Rolla, Sedalia, Chillicothe) than in the high fluoride cities (Fulton, Mexico, Columbia).

4. Dental caries rates were intermediate in the middle fluoride cities (Boonville, Jefferson City, Warrensburg).

5. An average of 57 percent of the children living in the high fluoride cities were caries-free (Fulton, Mexico, Columbia); compared to 18 percent of the children in the low fluoride cities (Rolla, Sedalia, Chillicothe).

6. Fluorides were present in the water supplies in amounts that did not esthetically affect the teeth of the children examined.

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APPENDIX TABLE 1.—*A comparative summary of the climatological data concerning the selected cities in the Missouri dental study*

City	Mean annual temperature	Avg. length growing season	Avg. date late killing frost (spring)	Avg. date early killing frost (fall)	Avg. annual rainfall and melted snow	Avg. annual snowfall	Avg. No. days rain .01" or more	Altitude in feet
Rolla	55.5	191	Apr. 16	Oct. 24	41.35	20.8	99	1,202
Sedalia	54.4	179	Apr. 20	Oct. 16	39.97	17.7	95	910
Chillicothe	53.8	170	Apr. 24	Oct. 11	36.22	22.2	93	771
Boonville	54.2	---	----	----	39.76	14.8	100	650
Jefferson City	54.8	188	Apr. 15	Oct. 20	38.02	26.7	94	557
Warrensburg	55.2	180	Apr. 14	Oct. 20	38.05	20.5	88	878
Fulton	54.8	181	Apr. 20	Oct. 18	38.12	17.9	97	818
Mexico	53.7	180	Apr. 20	Oct. 17	39.63	23.9	110	800
Columbia	55.0	189	Apr. 13	Oct. 19	39.08	19.8	111	778

City [1]	Annual mean humidity			
	12:30 a.m. (CST)	6:30 a.m. (CST)	12:30 p.m. (CST)	6:30 p.m. (CST)
Kansas City	72	77	56	50
Columbia	77	83	60	63
St. Louis	77	76	57	61

[1] Data regarding humidity of this area are available for these three cities only.

Sources U.S. Department of Commerce, Weather Bureau (*12, 13, 14, 15*).

Appendix Table 2.—*A record of the fluoride determinations in parts per million made on the water supplies of the study cities 1935–1952*

Year	Rolla	Sedalia	Chillicothe	Boonville	Jefferson City	Warrensburg	Fulton	Mexico	Columbia
	Annual mean of all fluoride determinations made 1935–1952[1]								
1935	----------	----------	----------	----------	----------	0.70	0.85	----------	1.30
1936	0.10	0.20	----------	0.40	0.60	.70	.80	0.70	1.30
1937	.10	.20	0.15	.45	.45	.55	.85	.83	1.10
1938	.10	----------	.45	.55	.55	----------	.80	.10	1.25
1939	----------	----------	----------	----------	----------	----------	----------	----------	----------
1940	----------	----------	----------	----------	----------	1.30	1.00	1.50	2.00
1941	----------	----------	----------	----------	----------	----------	----------	----------	----------
1942	.05	----------	----------	----------	----------	----------	.90	----------	2.20
1943	----------	----------	----------	----------	----------	----------	.90	----------	1.00
1944	----------	----------	----------	----------	----------	----------	----------	----------	1.20
1945	----------	----------	----------	----------	----------	.80	1.00	.90	1.20
1946	----------	----------	----------	----------	----------	.50	----------	----------	----------
1947	----------	----------	----------	----------	----------	.60	.80	.80	----------
1948	----------	----------	----------	----------	----------	----------	----------	.90	1.00
1949	----------	----------	----------	----------	----------	.60	.80	.90	1.20
1950	----------	----------	----------	----------	----------	.50	.90	1.00	1.50
1951	.09	.20	.25	.47	.50	.57	.87	.97	1.06
1952	.06	.13	.19	.25	.37	.64	.87	1.04	1.18
Average 1935–1952	.06	.18	.26	.42	.49	.68	.87	.87	1.32
	Record of fluoride determinations made during period of study								
October 1951	0.05	0.20	No Sample	0.50	0.50	0.50	0.90	1.00	1.00
November 1951	.05	.20	.30	.50	.50	.60	.80	1.00	1.25
December 1951	.05	.20	.20	.40	.50	.60	No Sample	.90	1.00
January 1952	.05	.10	.05	.40	.45	.60	do.	1.20	1.30
February 1952	.05	.05	.08	.25	.30	.55	.80	.90	.90
March 1952	.05	.05	.15	.15	.25	.55	.85	.95	1.10
April 1952	.05	.05	.20	.05	.30	.60	.70	1.00	1.00
May 1952	.05	.05	.25	.25	.30	.70	.80	1.00	1.25
June 1952	.10	Lab. Acc.	.20	.20	.40	.70	1.00	1.10	1.50
July 1952	No Sample	.20	.30	No Sample	.40	.70	.90	1.00	1.00
August 1952	.05	.25	.25	.30	.40	.70	.90	1.00	1.40
September 1952	.10	.25	.20	.40	.50	.70	1.00	1.20	1.20
Avg. Oct. 1951 to Sept. 1952	.06	.15	.20	.31	.40	.63	.86	1.02	1.16

[1] All determinations made by procedures given in *Standard Methods for the Examination of Water and Sewage*, 8th and 9th editions.

All determinations from October 1951 to September 1952 inclusive were made on monthly composites of weekly water samples. The tests were done by one operation using the Scott modification of the Sanchis B method. The Rolla determinations from October 1951 to September 1952 inclusive listed as 0.05 p.p.m. are below this concentration but determinations below 0.05 p.p.m. are beyond the limitations of the test itself.

Appendix Table 3.—*Summary of the source and treatment of the water supplies of the study cities. (Data obtained from the Bureau of Public Health Engineering, Missouri State Division of Health)*

City	Source of Water Supply	Treatment of Water Supply
Rolla	Five drilled wells: No. 1—Completed 1930, depth 1,710 ft., water bearing strata—Lamotte sandstone. No. 2—Completed 1935, depth 1,765 ft., water bearing strata—Lamotte sandstone. No. 3—Completed 1942, depth 1,175 ft., water bearing strata—Potosi dolomite. No. 4—Completed 1947, depth 1,078 ft., water bearing strata—Potosi dolomite. No. 5—Completed 1947, depth 1,150 ft., water bearing strata—Potosi dolomite. (In 1949, 1950 and 1951, approximately 80% of water used was from wells 3, 4 and 5.)	None.
Sedalia	Two surface water impoundments	Coagulation with alum and lime, activated carbon for taste and odor control, sedimentation, rapid sand filtration, post lime for pH control, and post chlorination.
Chillicothe	Grand River	Presedimentation, lime softening, recarbonation, coagulation with alum, sedimentation, rapid sand filtration, chlorine-ammonia disinfection to 1945, free residual chlorination from 1945 to present.
Boonville	Missouri River	Presedimentation, lime soda softening recarbonation, prechlorination, coagulation with alum, activated carbon for taste and odor control, sedimentation, rapid sand filtration, post-chlorine-ammonia disinfection.
Jefferson City	Missouri River	Presedimentation, lime softening, coagulation with ferric sulfate (sodium aluminate or alum occasionally), prechlorination, activated carbon for taste and odor control, sedimentation, rapid sand filtration, post-chlorine-ammonia disinfection.
Warrensburg	Four drilled wells: No. 1—Completed 1930, depth 1,075 ft., water bearing strata—Gunter sandstone and Eminence dolomite. No. 2—Completed 1930, depth 1,000 ft., water bearing strata—Gunter sandstone and Eminence dolomite. No. 3—Completed 1946, depth 1,050 ft., water bearing strata—Gunter sandstone and Eminence dolomite. No. 4—Completed 1947, depth 870 ft., water bearing strata—Gasconade dolomite. (Wells No. 1, and No. 2 abandoned in 1947).	Copper sulfate occasionally in open storage reservoir, disinfection with chlorine or chlorine ammonia (during summer), aeration 1939 to present.

Appendix Table 3.—*Summary of the source and treatment of the water supplies of the study cities. (Data obtained from the Bureau of Public Health Engineering, Missouri State Division of Health)*—Continued

City	Source of Water Supply	Treatment of Water Supply
Fulton	Four drilled wells: No. 1—Completed 1914, depth 1,121 ft., water bearing strata—Gunter sandstone. No. 2—Completed 1931, depth 1,140 ft., water bearing strata—Gunter sandstone. No. 3—Completed 1933, depth 1,350 ft., water bearing strata—Eminence dolomite. No. 4—Completed 1940, depth 1,270 ft., water bearing strata—Eminence dolomite. (Wells No. 2 and No. 3 abandoned in 1940, well No. 4 main source since then, well No. 1 used occasionally.)	Chlorination since 1944.
Mexico	(A) Three drilled wells and (8) surface water impoundments: (A) No. 1—Completed 1914, depth 1,213 ft., water bearing strata—Gasconade dolomite. No. 2—Completed 1924, depth 1,150 ft., water bearing strata—Gasconade dolomite. No. 3—Completed 1947, depth 1,200 ft., water bearing strata—Gasconade dolomite. No. 4—Completed 1948, depth 1,400 ft., water bearing strata—Gasconade dolomite.	(A) Iron removal and chlorination prior to 1950. Lime softening started 1950.
	(B) Impounded water used during rainy season only. Approximately 25% of total 1939–1943 and 15% of total 1943–1949. Impounded supply adandoned in 1950.	(B) Coagulation with alum and lime, sedimentation, chlorination prior to adandonment in 1950.
Columbia	Five drilled wells: No. 1—Completed 1935, depth 1,200 ft., water bearing strata—Proctor dolomite. No. 2—Completed 1927, depth 1,200 ft., water bearing strata—Proctor dolomite. No. 3—Completed 1928, depth 1,200 ft., water bearing strata—Proctor dolomite. No. 4—Completed 1947, depth 1,500 ft., water bearing strata—Proctor dolomite. No. 5—Completed 1948, depth 1,200 ft., water bearing strata—Proctor dolomite.	Aeration and chlorination started 1942.

Appendix Table 4.—*Chemical analyses of the finished water of the study cities. All determinations are in parts per million except the pH*

Date	Rolla	Sedalia	Chillicothe	Boonville	Jefferson City	Warrensburg	Fulton	Mexico	Columbia
	Oct. 9 1951	Oct. 22 1951	May 16 1951	May 16 1951	Jan. 15 1951	Nov. 15 1951	June 4 1951	Aug. 20 1951	Apr. 26 1951
pH	7.4	8.0	8.9	9.2	7.5	7.8	7.2	8.4	7.7
Total alkalinity ($CaCO_3$)	229.0	42.0	20.0	39.0	249.0	217.0	307.0	171.0	293.0
Pheno. alkalinity ($CaCO_3$)	0	0	4.9	9.8	0	0	0	0	0
Bicarbonates (HCO_3)	279.4	51.0	29.7	35.7	303.0	265.0	374.5	208.0	356.6
Carbonates (CO_3)	0	0	2.9	5.9	0	0	0	0	0
Silica (SiO_2)	6.0	2.0	4.0	5.0	8.0	6.0	8.0	6.0	8.0
Total iron	0.5	0.25	0.04	0.08	0.2	0.10	0.3	0.08	0.04
Aluminum	0.1	0.1	0.01	0.9	0.1	0.10	0.1	0.3	0.1
Sodium & Potassium (Na)	6.1	7.7	13.5	28.8	62.6	105.0	44.1	67.0	49.9
Calcium	48.5	17.5	47.2	36.7	37.8	59.5	67.1	12.8	50.7
Magnesium	31.9	4.8	4.6	4.5	20.5	26.9	30.6	28.9	30.1
Sulfates	22.8	25.9	101.2	95.3	24.3	32.1	40.9	64.4	30.5
Chlorides	5.0	6.3	18.1	21.0	27.3	155.4	21.0	41.6	26.0
*Fluorides									
Total residue	304.0	83.0	288.0	317.0	366.0	559.0	466.0	371.0	443.0
Loss by ignition	128.0	28.0	85.0	93.0	138.0	92.0	193.0	96.0	153.0
Hardness total ($CaCO_3$)	252.0	64.0	137.0	116.0	179.0	260.0	293.0	150.0	273.0
Hardness carbonate ($CaCO_3$)	229.0	42.0	29.0	39.0	179.0	217.0	293.0	150.0	273.0
Hardness non carbonate ($CaCO_3$)	23.0	22.0	108.0	77.0	0	43.0	0	0	0

APPENDIX TABLE 5.—*Distribution of the 3,206 children examined, according to age and sex, and classified by cities*

City	Total	M	F	Both Sexes	M	F	Both Sexes	M	F	Both Sexes
		Age in years last birthday								
		6			7			8		
Rolla	142	7	6	13	7	10	17	11	10	21
Sedalia	820	63	57	120	38	58	96	58	64	122
Chillicothe	262	22	17	39	17	14	31	26	31	57
Boonville	122	11	4	15	11	5	16	7	9	16
Jefferson City	671	34	45	79	48	52	100	44	36	80
Warrensburg	157	7	7	14	11	13	24	9	11	20
Fulton	202	18	14	32	18	17	35	15	14	29
Mexico	311	27	16	43	21	20	41	24	25	49
Columbia	519	34	39	73	26	38	64	37	29	66
		9			10			11		
Rolla		9	13	22	7	9	16	6	9	15
Sedalia		54	35	89	57	31	88	44	34	78
Chillicothe		14	16	30	23	15	38	13	8	21
Boonville		8	7	15	10	8	18	7	4	11
Jefferson City		54	50	104	37	42	79	34	39	73
Warrensburg		13	13	26	8	10	18	11	9	20
Fulton		11	8	19	8	12	20	8	9	17
Mexico		14	19	33	12	23	35	15	22	37
Columbia		32	40	72	28	25	53	35	25	60
		12			13			14		
Rolla		5	6	11	5	14	19	3	5	8
Sedalia		41	41	82	32	44	76	34	35	69
Chillicothe		11	6	17	11	8	19	2	8	10
Boonville		4	5	9	5	9	14	1	7	8
Jefferson City		42	40	82	26	25	51	10	13	23
Warrensburg		6	10	16	1	9	10	5	4	9
Fulton		10	7	17	7	9	16	7	10	17
Mexico		19	16	35	14	18	32	3	3	6
Columbia		27	22	49	21	20	41	18	23	41

APPENDIX TABLE 6.—*The dental caries experience in the permanent teeth of 3,206 white children age 6–14 years, classified by cities*

Cities	Average F. concentration in p.p.m.	Number of children examined	Classification of dental caries experience	Dental caries experience in the permanent teeth of 3,206 white childern age 6-14 years, and classified by cities								
				Age in years last birthday								
				6	7	8	9	10	11	12	13	14
				Average number of permanent teeth affected per child								
Rolla	0.08	142	Decayed	1.15	1.12	1.13	1.46	1.32	2.27	4.45	4.74	3.50
			Missing	------	.06	.10	.09	.75	.20	.64	.73	1.13
			Filled	------	1.00	1.10	2.18	2.31	2.06	3.91	3.21	6.37
			DMF	1.15	2.18	2.33	3.73	4.38	4.53	9.00	8.68	11.00
Sedalia	0.18	820	Decayed	.81	1.71	1.87	2.05	2.80	3.29	3.70	5.34	4.58
			Missing	.02	.11	.16	.62	.67	.67	.93	1.45	1.25
			Filled	.05	.22	.88	.97	1.31	1.90	2.02	2.58	3.49
			DMF	.38	2.04	2.91	3.64	4.76	5.92	6.65	9.37	9.32
Chillicothe	0.26	262	Decayed	.87	1.16	1.53	1.67	1.63	1.76	4.00	5.95	3.10
			Missing	------	------	.11	.13	.26	.43	.29	.47	.60
			Filled	.08	.29	.75	1.00	1.08	1.10	2.59	1.74	5.00
			DMF	.95	1.45	2.79	3.00	2.97	3.29	6.88	8.16	8.70
Boonville	0.42	122	Decayed	.13	.75	1.12	.34	.72	2.28	1.55	1.07	3.38
			Missing	------	------	.13	.13	.44	.45	------	.36	.25
			Filled	.07	.75	.88	1.20	.78	1.19	3.67	2.14	2.50
			DMF	.20	1.50	2.13	1.67	1.94	3.91	5.22	3.57	6.13
Jefferson City	0.49	671	Decayed	.45	.91	1.18	.84	.99	1.53	1.53	1.23	2.91
			Missing	------	------	.11	.13	.14	.19	.22	.18	.22
			Filled	.03	.25	.69	1.12	1.73	1.49	2.51	3.53	4.04
			DMF	.48	1.06	1.98	2.09	2.86	3.21	4.26	4.94	7.17
Warrensburg	0.68	157	Decayed	.43	.13	1.10	1.01	1.95	1.85	1.31	1.10	1.12
			Missing	------	------	------	.08	------	.15	------	.20	------
			Filled	------	.08	.05	.50	.72	.80	.63	2.60	1.44
			DMF	.43	.21	1.15	2.19	2.67	2.80	1.94	3.90	2.56
Fulton	0.87	202	Decayed	.40	.57	.24	.80	.40	.47	.29	.38	.70
			Missing	------	------	------	.05	.10	------	.24	------	.24
			Filled	.16	.23	.17	.47	.60	1.12	1.18	2.06	.41
			DMF	.56	.80	.41	1.32	1.10	1.59	1.71	2.44	1.35
Mexico	0.87	311	Decayed	.23	.46	.53	.67	.83	.32	.48	.97	3.50
			Missing	------	------	.02	.08	.14	------	.06	.25	.67
			Filled	.05	.05	.33	.33	1.20	.92	1.43	1.03	.33
			DMF	.28	.51	.88	1.06	2.17	1.24	1.97	2.25	4.50
Columbia	1.32	518	Decayed	.20	.25	.25	.44	.28	.43	.68	.95	.78
			Missing	------	------	------	.06	.05	.07	.10	.44	.12
			Filled	.01	.09	.45	.60	.79	.90	1.08	.93	.98
			DMF	.21	.34	.70	1.10	1.13	1.30	1.86	2.32	1.88

APPENDIX TABLE 7a.—*The prevalence of dental caries experience by percent in the deciduous dentition of 1,722 white children age 6–9 years, classified by cities*

Cities	Average F. concentration in p.p.m.	Number of children examined	The prevalence of dental caries experience in the deciduous dentition of 1,722 children according to age, classified by cities[1]			
			Age in years last birthday			
			6	7	8	9
			Percent of children affected by dental caries experience			
Rolla	0.08	73	100.0	88.2	100.0	95.5
Sedalia	.18	427	88.3	91.7	93.4	80.9
Chillicothe	.26	157	79.5	80.6	91.2	93.3
Boonville	.42	62	60.0	75.0	100.0	66.7
Jefferson City	.49	363	77.2	85.0	90.0	81.7
Warrensburg	.68	84	50.0	62.5	60.0	84.6
Fulton	.87	115	68.8	68.6	69.0	84.2
Mexico	.87	166	62.8	70.7	63.3	66.7
Columbia	1.32	275	54.8	53.1	60.6	61.1

[1] The prevalence of deciduous dental caries experience is confined to children age 6–9 years because of the normal exfoliation of these teeth.

Appendix Table 7b.—*The prevalence of dental caries experience in the permanent dentition of 3,206 white children age 6–14 years, classified by cities*

Cities	Average F. concentration in p.p.m.	Number of children examined	The prevalence of dental caries experience in the permanent dentition of 3,206 children according to age, classified by cities								
			Age in years last birthday								
			6	7	8	9	10	11	12	13	14
			Percent of children affected by dental caries experience								
Rolla	0.08	142	46.2	82.4	90.5	95.5	100.0	86.7	100.0	94.7	100.0
Sedalia	.18	820	37.5	77.1	85.2	91.0	94.3	94.9	93.9	97.4	98.6
Chillicothe	.26	262	51.3	58.1	82.5	86.7	76.3	76.2	100.0	100.0	90.0
Boonville	.42	122	20.0	75.0	81.3	60.0	72.2	61.8	88.9	85.7	87.5
Jefferson City	.49	671	24.1	47.0	72.5	74.0	82.3	83.6	86.6	88.2	100.0
Warrensburg	.68	157	21.4	12.5	50.0	65.4	72.2	60.0	62.5	90.0	77.8
Fulton	.87	202	21.9	37.1	27.6	52.6	45.0	64.7	59.8	62.5	70.6
Mexico	.87	311	20.9	26.6	40.8	48.5	62.9	45.9	57.1	62.5	66.7
Columbia	1.32	519	13.7	21.9	33.3	45.8	47.2	53.3	61.2	65.9	58.5

NATURAL FLUORIDATION IN JACKSONVILLE, FLA. Prevalence of Dental Caries, Tooth Mortality, and Fluorosis

William P. Kroschel, Frederick E. Hasty, Jr., Floyd H. DeCamp, and Wilson T. Sowder

Water consumption of an individual depends to an extent on factors such as mean annual temperature, seasonal variation, humidity, and elevation above sea level. There is relationship between the water consumption of an individual and the amount of fluoride that should be present in drinking water (*1–7*). The most effective fluoride concentration for a public water supply should be determined for each set of environmental conditions (*8*).

The desirable fluoride concentration for any community's water supply is that amount which affords its population maximum protection against dental caries, with the minimal possibility of mottled enamel, which occurs in the teeth of persons living in communities whose water contains excessive amounts of naturally occurring fluoride (*9, 10*).

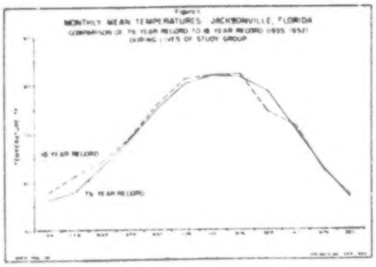
For the United States, fluoride concentration has been established at 1.0 to 1.5 parts per million, based primarily on epidemiologic studies of communities containing varying amounts of natural fluoride in their water supplies (*11*). These study areas are in the Middle West, where the climate may be considered cool and dry, with mean annual temperatures of 50° F. or less, and with considerable seasonal variations. A special committee of the American Water Works Association estimated that 0.70 parts fluoride per million parts water may be sufficient and desirable for regions where the mean annual temperature is 70° F. or higher (*12*). The need for epidemiologic studies in areas having more humid, warmer climates and naturally fluoridated water is apparent (*13*).

MATERIALS AND METHOD

Jacksonville, Florida, is as representative as many other cities along the southern seaboard, and for many years its municipal water supply has contained a uniform amount of natural fluoride. Detailed records of its climatic environment and water supply are available. This city affords an excellent opportunity for studying the relationship between fluoride and dental health in warm coastal regions of the southeastern seaboard of this country.

Geography.—Jacksonville (population 204,517) is located in the northeastern portion of the Florida Peninsula. The city is situated along the St. Johns River estuary at an elevation equal to sea level some 18 miles from the Atlantic Ocean.

Climate.—The annual mean temperature for the past 79 years has been 69.4° F., and for the past eight years it has been 70.4° F. For this same 79 year period there has been an annual mean seasonal variation of 25.5 degrees, seasonal temperatures ranging from a high of 82.0° F. in July to a low of 56.5° F. in January (fig. 1) (*14*).



A high annual rate of rainfall gives Jacksonville a definitely humid atmosphere. For the past 14 years, the average relative humidities have been: 87 percent at 1:30 a.m. (EST), 87 percent at 7:30 a.m., 55 percent at 1:30 p.m. and 74 percent at 7:30 p.m. (*15*).

WATER SUPPLY DATA

Source, Treatment, and Distribution.—For 72 years the Jacksonville Water Department has been the only public water supply for the city's population. Water has been supplied by free-flowing deep wells which are artesian in nature.

There are some 50 such wells, 45 of which are 1,000 feet or more in depth, that are scattered throughout the city's area. The deepest of these wells is 1,365 feet and the shallowest is 529 feet in depth (*16*). All water obtained from these wells is pumped to a central reservoir, and thence into the citywide distribution system. The only treatment received by the water is aeration to remove hydrogen sulfide as water enters the reservoir, and chlorination as it leaves the reservoir.

Fluoride Content.—Fluoride was first detected in Jacksonville's water in a series of analyses made July-September 1934. Seventeen samples were analyzed, including samples from individual wells and composites from the distribution system. Using the analytic methods of that era, fluoride concentrations were found in all samples which ranged from 0.55 parts per million to 0.75 parts per million. Distribution samples from the municipal supply were reported as having a fluoride content of 0.60 parts per million.

Later analyses of distribution samples in 1946 disclosed concentrations of 0.60 parts per million, and using newer methods, of 0.70 parts per million in 1949 and 1951. The same water-bearing aquifer, and also most of the same wells, have been in use since before fluoride was detected in 1934, and are used today.

These analyses, continuous use of same source wells, and similar fluoride contents of more recently drilled wells into the same aquifer are the basis for determining that the fluoride content of Jacksonville's water has been constant and uniform for more than 20 years (*17*).

DENTAL STUDY METHOD

A dental study was made to determine: (1) The extent of protection against dental caries afforded by continuous use of Jacksonville's naturally fluoridated waters (*18*) and (2) if this fluoride ion concentration is causing any harmful or esthetically objectionable dental fluorosis (mottled enamel).

Study Group.—Principals in all schools in the city distributed questionnaire cards to white children ranging in age from six through 17 years. Principals and classroom teachers in each school were impressed with the importance of distributing these cards to children of each socioeconomic level represented in their particular school. The objective of obtaining a thoroughly representative cross section of children in each age level was emphasized to these school officials.

After cursory screening, 21,000 children, nearly two thirds of all children in this age range, were given the questionnaire cards to be completed by their parents, containing these questions:

(1) Has your child lived within Jacksonville's city limits since birth?
(2) Has your child used the municipal water for drinking purposes since birth?
(3) Has your child *never* been absent from within the city limits for *more* than 30 days at one time?

Of the children thus sampled, there were 1,590 whose cards were returned by their parents answered "Yes." Of this number there were children in each age group from all of the schools, and school officials, county health department officials, and public health nurses confirmed that the study group was as representative of children of the city as possible who could meet the requirements for continuous exposure to the municipal water. The study group was composed of all white school children in the selected age range who had continuity of exposure to both the municipal water and the city's climate.

Dental Examination Procedure.—Each child was examined by a dentist in natural lighting using a No. 3 plain mirror and a No. 5 double end explorer. The clinical crowns of the permanent teeth (third molars excluded) were carefully examined for any carious lesion. All dental examinations in this study were performed by the same two examiners. Prior to the actual study, a special test group of 50 children was examined separately by each dentist. Findings were recorded separately, and later compared. When findings differed, the child was reexamined. Differences of interpretation of clinical evidence of caries were detected and reconciled. Diagnostic accord was thus reached between the two examiners.

Dean's "Classification of Mottled Enamel Diagnosis" was the criterion used to determine macroscopically the presence of dental fluorosis, and to diagnose its severity (*19*). Effort was made by the examiners to differentiate between fluorosis and other enamel dystrophies (*20*).

The dental examinations of each child covered four specific categories in permanent dentition.

(1) Number of decayed, missing and filled (DMF) teeth.
(2) Number of extracted teeth as result of caries experience only.
(3) Number of teeth indicated for extraction as result of caries experience.
(4) Fluorosis classification.

Examination findings were recorded on appropriate forms.

FINDINGS

Caries Prevalence in Permanent Teeth (Table 1).—Of the 1,590 children, 710 children, or 44.6 percent, had experienced no caries in permanent teeth. The distribution of these "caries-free" children by age groups is shown in table 1. Classification of "caries-free" means that the child had no active carious lesions, none that had been corrected by fillings, and no teeth missing as the result of caries, or, no DMF teeth. The DMF rates increased with age from 0.14 teeth at age six to 4.26 teeth at age 17.

Annual Caries Incidence.—The caries attack rate, determined by method of least squares fitted to a straight line, is 0.345. This figure, 0.345, represents the average number of permanent teeth per child becoming carious per year among the Jacksonville children, between ages six and 17 years, who had had continuity of use of the naturally fluoridated water since birth.

Permanent Tooth Loss.—Of the study group, 1,393 children, 87.6 percent, had no missing permanent teeth as the result of dental caries. The average rate of missing teeth per child varied from none at age six to 0.32 at age 17. Tooth mortality in the study group is shown in table 2.

Table 1.—*Caries prevalence in Jacksonville, Florida, children by age specific groups—permanent dentition*

Age	Number of children examined	Percentage of children caries-free	Average number of DMF teeth per child [1]
6	96	92.7	.14
7	158	81.0	.61
8	171	53.2	1.01
9	140	44.3	1.34
10	123	43.9	1.43
11	123	40.3	1.60
12	109	47.7	1.81
13	150	31.7	2.75
14	193	34.2	2.52
15	115	23.5	3.28
16	116	24.1	3.47
17	96	19.7	4.26
Totals	1,590	....	....

[1] DMF (Decayed, Missing and Filled) is a cumulative index of the entire caries experience of an individual representing the total of the number of permanent teeth that are: (1) Carious (D), (2) Missing as result of caries (M), and (3) Filled because of caries (F).

Fluorosis Experience.—There were 4 cases of "mild" dental fluorosis and 119 cases of "very mild" fluorosis. The 123 children with definite macroscopic evidence of fluorosis represent 7.5 percent of the study group. Of the remaining 92.5 percent, 182 children were classified as "questionable" and 1,285 children were "normal."

Dean (*19*) described "mild" fluorosis as: "The white opaque areas in the enamel of the teeth involve at least one-half of the tooth surface. The surface of molars, bicuspids, and cuspids subject to attrition show their white layers worn off and bluish shades of underlying

Table 2.—*Permanent tooth mortality in Jacksonville, Florida, children by age specific groups—permanent dentition*

Age	Percentage with no permanent tooth loss	Average number of missing permanent teeth [1] per child
6	100.0	0
7	100.0	0
8	97.0	.04
9	94.2	.03
10	93.5	.09
11	87.6	.16
12	79.8	.23
13	68.5	.20
14	87.0	.21
15	70.4	.30
16	82.7	.32
17	86.4	.32

[1] Includes teeth indicated for extraction.

enamel normal. Light brown stains are sometimes apparent on the superior incisors."

No case of greater severity than "mild" was observed. There were no cases in which brown stains or discoloration of enamel were found. There were no cases of fluorosis in which physical enamel defects or esthetically objectionable appearance of the teeth was observed.

Fine white striations, involving incisal edges, tips of cusps, and occlusal portions of tooth crowns were observed in "very mild" cases. White opaque areas were observed, which involved at least half the tooth surfaces of second molars, bicuspids, and cuspids, particularly in the "mild" cases.

Evaluation of Findings.—Data of caries and fluorosis prevalence gathered in this study were compared to similar data from Aurora, Ill. Aurora was one of the cities in which epidemiologic studies disclosed a sufficient amount of naturally occurring fluoride in its water supply to reduce effectively the incidence of dental caries without causing severe fluorosis (*11*). Dental examinations, screening of the study group for continuity of residence and exposure to the public water, technic of dental examinations, tabulation, and statistical analysis of findings were conducted in the same manner in both of these studies.

Data from the Aurora studies were used as a standard to which results of pilot studies on fluoridation in Grand Rapids, Michigan, were compared and evaluated (*21*). It was on the basis of studies in Aurora and neighboring cities with similar water-borne fluorides that the determination was first made that 1.0 parts per million concentrations would effect a caries reduction of 60 to 65 percent (*22*).

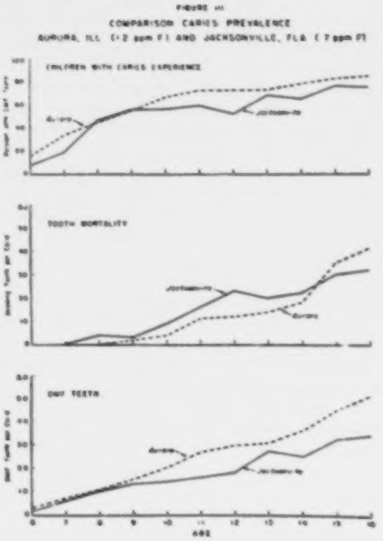
Geography and Climate.—Located in Northern Illinois, Aurora (population 50,576) is situated some 50 miles west of Chicago at an elevation of 678 feet above sea level. The annual mean temperature is 48.2° F. with a seasonal variation of 51.8 degrees (*23*). Humidity readings are not recorded in Aurora, but are available for the metropolitan Chicago area. Average summer humidities are 55 percent. Only summer humidities were recorded by the Chicago Weather Bureau during the time interval included in the lives of children in the Aurora study group. The Chicago Weather Bureau office stated that the humidity of this area is low enough to make the atmosphere rather dry, and heated air must be moistened for comfort (*24*).

Fluoride Content.—Aurora's water, obtained from deep wells, has a natural fluoride content determined to be uniform at 1.2 parts per million. This water was consumed by children in the dental study group with continuity of exposure from birth.

Caries Prevalence (Figure 3).—Caries prevalence, based on DMF rates, tooth mortality, and percentage of "caries-free" children, is lower in Jacksonville than in Aurora. By age 16, the average child in Jacksonville's study group had 1.68 fewer DMF teeth than a child of the same age in the Aurora study group. Permanent tooth mortality is essentially the same in both cities up to age 12, at which point Aurora's rises above Jacksonville's.

The percentage of children with no caries experience is higher in Jacksonville for the over-all study group and is higher in most specific age groups than in Aurora's.

Fluorosis Experience.—Less fluorosis of the "very mild" and "mild" types was observed in the Jacksonville study group. No cases of greater severity than "mild" were observed in either city. The incidence of fluorosis in Jacksonville is 13.0 percent compared to 15.0 percent in Aurora (*25*). Fluorosis experience for the study groups of both cities is shown in table 3.



SUMMARY

Dental findings of a study group of 1,590 white children, ages six through 17 years, having continuity of exposure since birth

Table 3.—*Comparison of fluorosis experience,[1] Aurora, Illinois[2] and Jacksonville, Florida: Incidence and distribution according to degree of affection*

Macroscopic Signs of Fluorosis	Aurora, Ill 1.2 p.p.m. F.	Jacksonville, Fla. 0.7 p.p.m. F.
	(A) Number	
Total number of children examined	633	[3] 453
Fluorosis Absent:		
Normal	337	350
Questionable	201	44
Fluorosis Present:		
Very mild	88	56
Mild	7	3
Moderate	0	0
	(B) Percent	
Total examined	100.00	100.00
Fluorosis Absent:		
Normal	53.2	77.3
Questionable	31.8	9.7
Fluorosis Present:		
Very mild	13.9	12.4
Mild	1.1	0.6
Moderate	0	0
Incidence of affection	15.0	13.0

[1] Dean's "Classification of Mottled Enamel Diagnosis," pages 398-400 of Gordon's "Dental Science and Dental Art."

[2] Aurora data contained in Dean, H. T.; Jay, P.; Arnold, F. A., Jr., and Elvove, E.; Domestic Water and Dental Caries II, Pub. Health Rep. *56*: 15, Apr. 11, 1941, table 9.

[3] Fluorosis in Jacksonville as it occurred in the age groups 12–14 years compared here because these were the ages examined in the Aurora study group.

to Jacksonville's naturally fluoridated water (0.60–0.70 parts per million fluoride), and comparison of these findings with data obtained in a similar manner from Aurora, Illinois (1.2 parts per million fluoride) suggest these conclusions:

1. The naturally occurring fluoride content, 0.60 to 0.70 parts per million, of Jacksonville's communal water supply is providing a protection against dental caries for persons with continuous use of this water during the calcification of their permanent dentitions.

2. The degree of caries reduction effected appears to be as adequate as any yet found in other communities whose public water supplies contain higher concentrations of fluorine.

3. Since no objectionable or harmful fluorosis has resulted, no need to *decrease* the natural fluoride content of Jacksonville's communal water is indicated.

4. On the basis of present knowledge of the effectiveness of water fluoridation, there is no reason to believe that any increase in the present fluoride content of Jacksonville's communal water would effect any greater protection against dental caries.

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EFFECT OF NATURAL FLUORIDES ON CARIES INCIDENCE IN THREE GEORGIA CITIES

Fred D. Lewis, Jr. and Ernest C. Leatherwood, Jr.

Dental health services of the Georgia Department of Public Health and of the Public Health Service in Region IV were requested by the dental society, chamber of commerce, and health department of Savannah, Ga., to make a detailed study of the prevalence of dental caries in the children of Savannah in relation to the fluoride content of its water supply. The natural fluoride content of Savannah's water supply was approximately one-half the concentration recommended by the State health department. The question was: How much additional reduction in the caries attack rate would be derived from the adjustment of this concentration to the approved range?

A dental survey was planned for Savannah and two geographically comparable communities having known concentrations of natural fluoride in their public water system. It was estimated that Savannah had from 0.3 p.p.m. to about 0.5 p.p.m. fluoride concentration. The Georgia cities of Macon and Moultrie were chosen as controls, for, according to the water control laboratory of the State health department, the natural fluoride content of Macon's water supply was 0.11 p.p.m. and Moultrie's was about 0.75 p.p.m. These concentrations were determined from water samples collected during a 3-year period, 1954–56. (Macon obtains its water from the Ocmulgee River, Savannah, from 11 deep wells and the Savannah River, and Moultrie from 3 deep wells.) Forty samples tested for Macon showed a range of 0.0 to 0.5 p.p.m. fluoride in the water supply. Moultrie's range was 0.3 to 1.4 p.p.m. in 52 water samples tested.

Macon would serve as the baseline while Moultrie would indicate the caries attack rates present at the lowest fluoride level recommended by the State health department. The optimum fluoride concentration as determined by the formula relating fluid intake to mean maximum temperature (*1*) approximates the natural fluoride concentration present in the Moultrie water system. The mean maximum temperature according to the U.S. Weather Bureau station in Atlanta and Athens, Ga., was 76.6° F. for Savannah, 77.6° F. for Macon, and 80° F. for Moultrie, over a period of 82, 57, and 24 years respectively.

Over the years, Savannah has continually expanded, outreaching and overtaxing the public water system. To meet the increased demands, new wells were added directly to the water system at the point of greatest need. Today there are 11 independent deep wells and a surface supply with 2 separate pumps. Between March and October 1956, 138 samples from all water sources showed a range in fluoride of 0.0 to 0.8 p.p.m. The average fluoride concentration for each well range from approximately 0.31 p.p.m. to 0.53 p.p.m., while the surface supply averaged about 0.06 p.p.m. This resulted in an overall numerical average of about 0.37 p.p.m. fluoride concentration (table 1). Since records have been kept only in recent years on the fluoride concentration in the water system, the assumption was that the samples taken in 1956 would be indicative of the fluoride concentration in the water system for the entire life of the children examined.

The pumpage rate of each well fluctuated, depending upon the needs of the system, and since there was no common reservoir, there was no distribution constant. During 1956, the pumpage rate of individual wells varied from approximately 28 million to 1.4 billion gallons of water, and the surface source supplied about 35 million gallons. The inconstant pumpage rate and variation in the fluoride concentration necessitated the determination of a weighted average fluoride concentration in the water system for the year. By dividing the total parts fluoride from each well by the total number of gallons pumped, a concentration of 0.38 p.p.m. was calculated.

MATERIALS AND METHODS

A survey technique to obtain the required data was decided upon early in the planning stage (*2*). Participation in the dental survey was limited to white school children 6–15 years of age who were continuous residents, had used only the public water supply since birth, and had parental consent.

Careful dental examinations were conducted by two dentists, Hubert H. Martin from the Georgia Department of Public Health and Ernest C. Leatherwood from Region IV, Public Health Service. A tooth surface was considered carious if the end of the dental explorer penetrated the soft, yielding tooth structure; if, in the case of pits and fissures, the explorer caught, supported its own weight, and met resistance when it was withdrawn; or if the surface had a carious lesion which was clinically obvious. The observations of the two examining dentists were recorded in code on the patient's chart (*3*) along with the following data:

1. Number of teeth erupted.
2. Number of permanent teeth extracted and reason for extractions: orthodontic purposes, trauma, or otherwise.
3. Number of primary and permanent teeth needing extraction.
4. Number of unfilled carious teeth and number of surfaces involved.
5. Number of filled teeth and number of surfaces restored.

FINDINGS

A total of 9,301 school children participated in this dental survey, 3,890 from Macon, 4,871 from Savannah, and 540 from Moultrie. Even though the number of children in Moultrie was relatively small, when the data from all three communities on caries-free children and on decayed, missing, and filled (DMF) teeth were subjected to the chi-square test, they were found to be statistically significant (*4*).

Table 2 shows the number of children examined as well as the percentage with caries-free permanent teeth. The total for all ages shows that for permanent teeth, 17.1 percent of the children in Macon are caries-free, while 23.3 percent of Savannah's children are caries-free, and 44.3 percent of Moultrie's children are caries-free.

TABLE 1.—*Average fluoride concentration in water supply, Savannah, Ga., 1956*

Well No.	Gallons of water pumped	Number test samples	Fluoride range (ppm)	Average fluoride concentration (ppm)
1	220, 794, 332	11	0. 4–0. 8	0. 53
2	317, 192, 899	11	. 3– . 7	. 47
3	28, 474, 120	4	. 3– . 5	. 42
4	1, 059, 072, 217	11	. 3– . 5	. 45
5	1, 389, 495, 413	14	. 3– . 5	. 34
6	407, 671, 408	14	. 2– . 4	. 31
7	404, 160, 621	10	. 2– . 7	. 35
8	1, 112, 522, 738	9	. 3– . 4	. 32
9	788, 729, 221	13	. 3– . 5	. 39
10	596, 650, 207	10	. 3– . 5	. 38
11	179, 933, 182	7	. 3– . 5	. 41
12 and 13 [1]	37, 398, 000	24	. 0– . 3	. 06
Total	6, 542, 094, 358	138	. 0– . 8	. 37

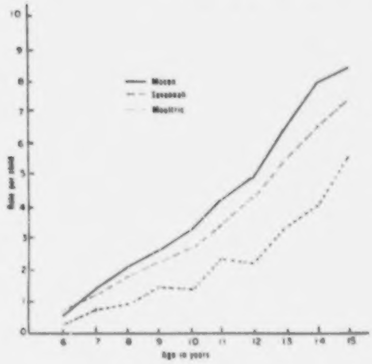
[1] Surface water supply.

Table 2.—*Percentage of caries-free permanent teeth in children, by age, in Macon, Savannah, and Moultrie, Ga., 1956–57*

Age (years)	Number of children examined			Percent children caries-free		
	Macon	Savannah	Moultrie	Macon	Savannah	Moultrie
6	371	554	71	48. 2	49. 3	76. 1
7	419	622	80	40. 3	42. 1	58. 8
8	413	652	61	25. 7	29. 4	50. 8
9	442	638	64	18. 8	23. 7	40. 6
10	402	478	64	10. 0	16. 9	46. 9
11	280	440	40	10. 0	13. 9	27. 5
12	300	417	37	6. 3	9. 4	32. 4
13	432	374	47	5. 1	9. 6	27. 7
14	450	397	52	3. 1	5. 5	21. 2
15	381	299	24	1. 8	6. 0	16. 7
Total	3, 890	4, 871	540	17. 1	23. 3	44. 3

The percentage difference in the total DMF rates, as shown in the last three columns of table 3, indicates that the children of Savannah have 35.6 percent fewer caries than those of Macon.

Decayed, missing, or filled permanent teeth per child by age, Macon, Savannah, and Moultrie, Ga., 1956–57



Moultrie shows 60.8 percent less dental decay than Macon and 39.1 percent less than Savannah.

The difference in the decayed, missing, or filled rate per child at each age in the three cities is shown in the chart.

An analysis of the decayed-filled (df) rate in primary teeth is presented for these cities in table 4. Moultrie has the lowest df rate for the children examined.

DISCUSSION

From the findings, it is obvious that an inverse relationship exists between the caries attack rates and the concentration of fluorides in the water system of these three communities. Approximately one-fourth of the permanent teeth of the children examined in Macon were decayed, missing, or filled, one-fifth in Savannah, and one-ninth in Moultrie.

The DMF rates of the 12–14-year-old groups in Macon, Savannah, and Moultrie can be plotted upon Dean's graph of dental caries experience in 21 cities (*5*). The pattern is similar even though these rates occur in cities with a warm climate.

The DMF percentage difference between Savannah and Moultrie demonstrates decisively the additional protection against dental decay that could be achieved in Savannah by increasing the fluoride concentration of the water system from 0.38 p.p.m. to at least 0.75 p.p.m. (but no more than 1.0 p.p.m.).

CONCLUSION

Savannah, with a natural fluoride content of about 0.38 p.p.m. in the water system, is providing its children with 35.6 percent protection against dental decay. An additional 39.1 percent reduction in caries would be expected if Savannah were to increase the fluoride concentration to equal Moultrie's (0.75 p.p.m.). Macon, with only a trace of fluoride in its water system, would expect to reduce dental caries in its children by at least 60.8 percent with adequate fluoridation.

SUMMARY

A dental survey of 9,301 continuously resident, white school children between the ages of 6 and 15 years was conducted in the cities of Macon, Savannah, and Moultrie, Ga., in order to determine the effect of different concentrations of natural fluoride in the water systems upon the rate of dental decay.

The natural fluoride concentration was approximately 0.11 p.p.m. for Macon, 0.38 p.p.m. for Savannah, and 0.75 p.p.m. for Moultrie. The mean maximum annual temperature for these cities was 77.6° F., 76.6° F., and 80.0 F. respectively.

On a percentage basis, Moultrie had about twice as many caries-free children as Savannah and more than two and one-half times as many as Macon.

The decayed, missing, or filled (DMF) rate for permanent teeth per child was 4.64 in Macon, 2.99 in Savannah, and 1.82 in Moultrie.

Moultrie's DMF rate was 60.8 percent lower than Macon's and 39.1 percent lower than Savannah's; Savannah's was 35.6 percent lower than Macon's.

The decayed-filled rates for primary teeth were slightly lower in Moultrie than in the other two communities.

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Table 3.—*Decayed, missing, or filled (DMF) permanent teeth, DMF rate per child, and percentage difference in DMF rate per child, by age, Macon, Savannah, and Moultrie, Ga., 1956–57*

Age (years)	Number permanent teeth DMF [1]			DMF rate per child			Percent difference in DMF rate		
	Macon	Savannah	Moultrie	Macon	Savannah	Moultrie	Savannah over Macon	Moultrie over Savannah	Moultrie over Macon
6	197	365	16	0. 53	0. 66	0. 23	+24. 5	−65. 2	−56. 6
7	581	733	70	1. 39	1. 18	. 88	−15. 1	−25. 4	−36. 7
8	837	1, 125	59	2. 03	1. 73	. 97	−14. 8	−43. 9	−52. 2
9	1, 127	1, 356	91	2. 55	2. 13	1. 42	−16. 5	−33. 3	−44. 3
10	1, 264	1, 236	83	3. 14	2. 59	1. 30	−17. 5	−49. 8	−58. 6
11	1, 158	1, 440	91	4. 14	3. 27	2. 28	−21. 0	−30. 3	−44. 9
12	1, 451	1, 733	80	4. 84	4. 16	2. 16	−14. 0	−48. 1	−55. 4
13	2, 663	1, 981	154	6. 16	5. 30	3. 28	−14. 0	−38. 1	−46. 8
14	3, 597	2, 464	208	7. 99	6. 21	4. 00	−22. 3	−35. 6	−49. 9
15	3, 182	2, 149	131	8. 35	7. 19	5. 46	−13. 9	−24. 1	−34. 6
Total	16, 057	14, 582	983	4. 64	2. 99	1. 82	−35. 6	−39. 1	−60. 8

[1] Exclusive of third molars.

Table 4.—*Number of primary teeth decayed or filled (df) and df rate for ages 6–11 years, Macon, Savannah, and Moultrie, Ga., 1956–57*

Age (years)	Number of children examined			Number of df primary teeth			df rate		
	Macon	Savannah	Moultrie	Macon	Savannah	Moultrie	Macon	Savannah	Moultrie
6	371	554	71	1, 770	2, 253	260	4. 77	4. 07	3. 66
7	419	622	80	2, 004	2, 455	299	4. 78	3. 95	3. 74
8	413	652	61	1, 890	2, 437	214	4. 58	3. 74	3. 51
9	442	638	64	1, 587	1, 941	157	3. 59	3. 04	2. 45
10	402	478	64	1, 029	960	116	2 56	2. 01	1. 81
11	280	440	40	301	486	23	1. 08	1. 10	0. 58
Total	2, 327	3, 384	380	8, 581	10, 532	1, 069	3. 69	3. 11	2. 81

A DENTAL CARIES AND FLUORIDE STUDY OF NINETEEN NEBRASKA CITIES

By Charles J. Gillody, Harold W. Heinz, and Paul W. Eastman, Jr.

INTRODUCTION

A successful determination of the scope of a disease and the effective application of measures for its control depend to a great extent upon knowledge of the manner in which the disease occurs in population groups. Therefore, intelligent application of methods for measuring the size of the problem and the control of dental caries requires the knowledge of where, when, and under what conditions the disease appears.

A study of this type was, of necessity, a cooperative multi-discipline endeavor engaged in by the Nebraska State Department of Health, Dental Committeemen of the Nebraska State Dental Association, School and Health authorities through the State, and the Public Health Service Regional Office VI staff.

THE STUDY

In an attempt to satisfy the prerequisites of knowledge of the manner in which the disease occurs in population groups, and more specifically the knowledge of where, when, and under what conditions the disease appears, this study was developed on the basis of a three-fold purpose as follows:

1. To determine the prevailing need—the amount of dental caries existing in the mouths of a selected group of Nebraska children at the time of the examination.
2. To determine if there is an inverse relationship between fluoride content of drinking water and dental caries.
3. To determine the optimum fluoride concentration for use in cities of this State if a positive relationship between fluoride and dental caries is established.

Several studies of the fluoride-dental caries relationship have been completed in other areas of this country (*1,2,3*) from which pertinent information can be obtained, nevertheless, there has not been a study of this type performed in Nebraska. Since it has been reported (*4*) that temperature affects the fluoride-dental caries relationship to some extent, it was decided that the study of the effects of fluorides on dental caries should be performed on children living in cities lying within certain designated parallels of latitude as modified by the range of average yearly maximum temperatures based on the entire period on record for this State.

The Study Groups

One thousand one hundred and sixty three continuous resident white children ages 7, 10, and 13 years received dental examinations on the basis of answers to resident and drinking water history questionnaires. Required of the children were verified continuity of residence from birth and continuous use of the public water supply as their regular source of drinking water. Children who had lived away from the city of their birth more than 3 months of any one year were not considered as continuous residents. Children who had received topical fluoride treatments were not included in the study.

The Study Cities

The cities included in the study were selected on the basis of a 14-year history of the public water supplies, the natural fluoride content of each water source, geographic distribution of the population, and the average yearly maximum temperatures.

All cities lie in an area between the 39° and 43° parallels of latitude. Climatologically, all cities are within a range of 67° and 76° Fahrenheit average yearly maximum temperatures. Four small cities in northwest Kansas, near the southwest border of Nebraska, which have high fluoride-bearing water supplies and lie within the above-mentioned ranges of latitude and temperature, were included in this study because there was an insufficient number of children living in the high natural fluoride cities of Nebraska to produce a statistically sound sample.

THE WATER SYSTEMS

Although each of the cities has natural fluoride in its water supply, the concentrations are different. All reliable fluoride determinations in the period from 1939 to 1954 (years during which the teeth of the sample children were developing) were used in computing the fluoride averages for the cities.

After verification of water histories and fluoride determinations, the 23 cities were grouped according to average fluoride content of their water supplies as follows: 9 low fluoride cities with averages ranging from 0.1 to 0.3 p.p.m.; 1 medium fluoride city with an average of 0.5 p.p.m.; and 13 high fluoride cities with averages ranging from 0.8 to 1.7 p.p.m. (See table 1.)

In computing the average fluoride concentration for each of the three groups of cities, the average fluoride concentration for each city was weighed according to the number of children examined. Accordingly, the nine low fluoride cities have an adjusted average fluoride determination of 0.2 p.p.m.; the 13 high fluoride cities have an adjusted fluoride determination of 1.1 p.p.m. Only one city having an average fluoride determination of 0.5 p.p.m. fell in the medium fluoride group hence no adjustment was necessary.

Table 1.—*Summary of the fluoride determinations made on the water supplies between 1939 and 1954 of the selected cities in the Nebraska Dental Study*

City	City Range of F. Determinations 1939 - 1954 P.P.M.	City Average F. Determinations 1939 - 1954 P.P.M.	Group Average F. Determinations 1939 - 1954 P.P.M.
Crete	0.1-0.3	0.2	
Falls City	0.2-0.3	0.2	
Holdrege	0.1-0.2	0.2	
Plattsmouth	0.2-0.6	0.3	
Schuyler	0.2-0.3	0.2	0.2*
Seward	0.1-0.2	0.1	
Wahoo	0.2-0.3	0.2	
Wayne	0.0-0.2	0.1	
York	0.2-0.3	0.3	
Omaha	0.1-0.6	0.5	0.5**
Alliance	0.6-1.3	0.9	
Arapahoe	0.7-0.8	0.8	
Benkelman	1.2-1.8	1.6	
Haigler	0.9-1.0	0.9	
Indianola	0.8-1.0	0.8	
Oakland	0.5-1.0	0.8	
Stratton	1.1-1.2	1.2	1.1*
Trenton	1.0-1.2	1.1	
Wauneta	0.7-1.0	0.8	
Atwood, Kansas	1.3-1.6	1.5	
Colby, Kansas	1.3-1.7	1.5	
Goodland, Kansas	1.1-1.6	1.3	
St. Francis, Kansas	1.4-1.8	1.5	

*Adjusted on the basis of the relative numbers of children examined.

**Adjustment unnecessary—Page 4, Paragraph 1.

All the cities except Omaha have well water supplies. Omaha obtains it water from the Missouri River. The history of the wells in each individual city was investigated to verify that they obtained water from the same aquifer throughout the period in which the teeth of the sample children were forming. This verified water history together with the available chemical analyses was considered strong evidence that there had been no signficant changes in chemical quality, including fluoride concentration, of the well water supplies during the 14-year period, 1939-1954.

Monthly fluoride determinations were available for a considerable time from the Omaha supply which indicated month to month variations (0.1 to 0.6 p.p.m.), but little variation in the yearly averages. The maximum fluoride determination at Plattsmouth (0.6 p.p.m.) is out of the range for the low fluoride cities. There is no explanation for this single extreme variation, however, it had relatively little influence on the average concentration for this city. More detailed information about the source and treatment of the different water supplies is given in table 5.

CRITERIA OF DENTAL CARIES AND THE DMF SYSTEM (*2*)

A tooth was considered to be carious if the lesion was clinically obvious, if the opacity of the enamel indicated underlying caries, or if either end of a No. 5 double-end dental explorer could be made to penetrate into soft yielding tooth structure. Pits or fissures which did not present one of the above criteria were not considered to be carious even though the dental probe could be made to catch.

Permanent Teeth—Permanent teeth were classified in regard to dental caries experience. Each tooth with positive caries experience was classified as either *decayed, missing,* or *filled* and scored as one against the patient. The total score for each patient was the sum of the decayed, missing, and filled teeth. A tooth which was both carious and filled was recorded as carious. No tooth was scored more than once. Teeth which were missing or restored as a result of a reported traumatic injury, extracted for orthodontic reasons or congenitally missing, and third molar teeth were not recorded.

Deciduous Teeth—Classification of dental caries in the deciduous teeth was made with respect to the entire dentition rather than individual teeth. The entire deciduous dentition was recorded as a plus, a minus, or a zero. A plus indicated the presence of one or more decayed or filled deciduous teeth; a minus indicated no caries experience in the deciduous teeth present; and a zero indicated that no deciduous teeth were present.

FINDINGS

The distribution of the sample was as follows:

523 children in the low fluoride cities (0.2 p.p.m.);

336 children in the medium fluoride city (0.5 p.p.m.);

304 children in the high fluoride cities (1.1 p.p.m.).

Although the number of children in each age group varied because of the continuous resident requirements, the numbers examined in all age groups were large enough to be significant. (See table 2.)

Dental Caries Prevalence—In the data obtained, an inverse relationship was found between the total dental caries experience rates and the average fluoride determinations of the groups of cities. This finding supports previous studies of the fluoride-dental caries relationship.

The decayed, missing and filled rates for permanent teeth among the low, medium and high fluoride groups were markedly different. In the low fluoride group, the sample children experienced an average of 1.90 DMF teeth at 7 years of age; 3.95 DMF teeth at 10 years of age; 6.93 DMF teeth at 13 years of age. Whereas, in the high fluoride group the caries experience averaged 0.33 DMF teeth in the 7 year olds; 1.52 in the 10

TABLE 2.—*Summary of the distribution of the 1,163 white children examined, according to age and classified by cities having low, medium and high natural fluoride-bearing water supplies. Nebraska Dental Study 1954*

City	Total	Number of Children by age last birthday		
		7	10	13
LOW FLUORIDE CITIES (Avg. F. 0.2 P.P.M.)*				
Crete	57	29	12	16
Falls City	104	46	41	17
Holdrege	62	38	17	7
Plattsmouth	64	26	23	15
Schuyler	45	19	15	11
Seward	40	17	13	10
Wahoo	49	19	17	13
Wayne	23	10	5	8
York	79	39	23	17
Total	523	243	166	114
MEDIUM FLUORIDE CITIES (Av. F. 0.5 P.P.M.)				
Omaha	336	131	96	109
HIGH FLUORIDE CITIES (Avg. F. 1.1 P.P.M.)*				
Alliance	110	54	40	16
Arapahoe	11	5	3	3
Benkelman	26	14	4	8
Haigler	10	2	4	4
Indianola	6	1	3	2
Oakland	24	10	8	6
Stratton	4	3	1	0
Trenton	13	8	3	2
Wauneta	9	5	2	2
Atwood, Kansas	24	0	10	14
Colby, Kansas	7	0	0	7
Goodland, Kansas	48	0	27	21
St Francis, Kansas	12	0	9	3
Total	304	102	114	88

year olds; and 2.53 in the 13 year olds. The medium fluoride group of children experienced a DMF average between the averages for the high and low fluoride groups. (See table 3.)

There is a marked difference in the percentage of children whose permanent teeth were affected by dental caries in the different groups of cities. In the low fluoride cities 74.1, 93.4, and 97.4 percent of the 7, 10, and 13-year-old children, respectively, were affected by dental caries. In the high fluoride cities, 19.6, 61.4, and 68.2 percent of the 7, 10, and 13 year olds, respectively, were affected. The percentage of children affected in the medium fluoride city was between that for the high and low fluoride groups. (See table 4.)

Dental caries prevalence was determined for deciduous teeth of 7-year-old children with respect to the entire dentition rather than individual teeth. Since the exfoliation time for deciduous teeth varies considerably among individuals, it is of doubtful value to compare missing rates in the primary dentition.

Of the children living in the low fluoride group of cities 89.3 percent were affected by dental caries of deciduous teeth. In the medium fluoride city 79.4 percent and in the high fluoride cities only 65.7 percent were affected in that respect. In the low fluoride cities only 11 percent were caries free while 34 percent were caries free in the high fluoride cities. (See table 4.)

FLUOROSIS

No evidence of dental fluorosis was observed in the low or medium fluoride groups of cities. Children of the sample group living in high fluoride cities with an average fluoride content of 1.4 p.p.m. and above exhibited very mild dental fluorosis. Only in the City of Benkelman, Neb., whose water supply contained an average of 1.6 p.p.m. fluoride was mottling of the enamel of three children noticeable to others than the dental profession. These three children, however, did not consider their teeth as being anything but pretty even though there was a slight spotty white discoloration to the enamel. Except in these three cases there was no aesthetic significance of this degree of fluorosis.

SUMMARY

1. (a) *Comparison of DMF Experiences for all Ages Between the High and Low Groups of Fluoride Cities*—There are approximately three times as many permanent teeth attacked by caries in the low fluoride cities as in the high fluoride cities. (See table 3.)

(b) *Comparison of the D, M, and F Experiences Between the High and Low Fluoride Groups of Cities*—In comparing the differences between caries experience of permanent teeth for all age groups of low and high fluoride cities those children living in the low fluoride cities have an average of approximately: three times as many decayed teeth; four times as many missing teeth; and two times as many filled teeth.

2. At the time of examination, the 7-, 10-, and 13-year-old children of the low fluoride areas, as compared to the children of the high fluoride areas, had an accumulated dental caries experience of permanent teeth as follows: 7-year-old children have on an average approximately six times as many DMF teeth; 10- and 13-year-old children have on an average approximately three times as many DMF teeth.

3. There is a marked difference in the percentage of children with caries free permanent teeth in the different groups of fluoride cities. In the low fluoride cities 26 percent, 7 percent and 3 percent of 7-, 10-, and 13-year-old children, respectively, were free of caries, whereas, in the high fluoride cities 80 percent, 39 percent and 32 percent of the 7-, 10-, and 13-year-old children, respectively, were caries free. (See table 4.)

4. Thirty four percent of the 7-year-old children living in high fluoride cities had caries free deciduous teeth, whereas only 11 percent were caries free in the low fluoride cities. (See table 4.)

CONCLUSIONS

1. Dental caries prevalence bears an inverse relationship to the average fluoride determinations of the groups of cities.

2. The data obtained support previous studies of the fluoride-dental caries relationship.

3. In view of the low DMF experience and the absence of fluorosis in the children living in the study cities having 1.0 to 1.3 p.p.m. fluoride-bearing water supplies, it was determined that a range of 1.0 to 1.3 p.p.m. should be maintained for all cities in Nebraska.

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TABLE 3.—*Summary of the dental caries experience in the permanent teeth of 1,163 white children ages 7, 10, and 13 years, classified by cities having low, medium and high natural fluoride bearing water supplies. Nebraska Dental Study 1954*

Classification of cities	No. of Children examined	Dental caries experience in permanent teeth, by age last birthday															
		7				10				13				Total			
		D	M	F	DMF	D	M	F	DMF	D	M	F	DMF	D	M	F	DMF
LOW FLUORIDE CITIES: (0.2 P.P.M. F.) Crete, Falls City, Holdrege Plattsmouth, Schuyler, Seward, Wahoo, Wayne, York	523	1.46	0.02	0.42	1.90	2.40	0.17	1.38	3.94	3.17	0.30	3.46	6.93	2.14	0.13	1.39	3.65
MEDIUM FLUORIDE CITIES: (0.5 P.P.M. F.) Omaha	336	0.79	0.01	0.33	1.13	1.23	0.08	1.39	2.70	1.79	0.10	3.02	4.91	1.24	0.06	1.50	2.80
HIGH FLUORIDE CITIES: (1.1 P.P.M. F.) Alliance, Arapahoe, Benkelman, Haigler, Indianola, Oakland, Stratton, Trenton, Wauneta, Atwood, Colby, Goodland, St. Francis	304	0.20	0.00	0.13	0.33	0.80	0.04	0.68	1.52	0.89	0.06	1.58	2.53	0.63	0.03	0.75	1.41

TABLE 4.—*Percent of children having dental caries experience in deciduous and permanent dentitions. 1,163 white children ages 7, 10, and 13 years, classified by cities having, low, medium and high natural fluoride-bearing water supplies. Nebraska Dental Study 1954*

Classification of Cities	Percent of children with caries experience, by age last birthday					
	Deciduous dentition		Permanent dentition			
	Children Examined	Age 7	Children Examined	Age 7	Age 10	Age 13
LOW FLUORIDE CITIES (0.2 P.P.M.)*	243	89.3	523	74.1	93.4	97.4
MEDIUM FLUORIDE CITIES (0.5 P.P.M.)*	131	79.4	336	48.1	83.3	89.9
HIGH FLUORIDE CITIES (1.1 P.P.M.)*	102	65.7	304	19.6	61.4	68.2

*Adjusted for the relative numbers of children examined.

TABLE 5.—*Summary of the source and treatment of the water supplies of the study cities*

City	Source of water supply	Treatment of water supply
Alliance	Six drilled wells; all in unconsolidated sand water-bearing formation; depths: No. 1, 120 feet, No. 2, No. 3, No. 4, No. 5, and No. 6—300 to 350 feet.	Stabilization with sodium hexameta-phosphate since about 1943.
Benkelman	Eight drilled wells, all in unconsolidated sand and gravel water-bearing formation; depths 30 to 52 feet.	None.
Oakland	Two drilled wells; both in Dakota sandstone water-bearing formation; depths approximately 350 feet.	Iron and manganese removal with aeration, sedimentation and rapid sand filtration.
Trenton	Three wells; all in unconsolidated sand and gravel water-bearing formation; depths approximately 40 feet.	None.
Arapahoe	Five drilled wells; all in unconsolidated sand and gravel water-bearing formation; depths approximately 75 feet.	Do.
Wauneta	Three drilled wells; all in unconsolidated sand and gravel water-bearing formation; depths 70 to 75 feet.	Do.
Indianola	One drilled well; unconsolidated sand and gravel water-bearing formation; depth 57 feet. (Use Bureau of Reclamation well, approximately same depth and formation, in emergencies.)	Do.
Stratton	Three drilled wells; all in unconsolidated sand and gravel water-bearing formation; depths 30 to 45 feet.	Intermittent disinfection with chlorine.
Haigler	Two drilled wells; both in unconsolidated sand and gravel water-bearing formation; depths approximately 90 feet.	None.
Omaha	Missouri River	Presedimentation, primary coagulation with alum and lime, secondary coagulation with alum, primary disinfection with chlorine, occasionally activated carbon for taste and odor control, rapid sand filtration, final disinfection with chlorine.
York	Nine drilled wells; all in unconsolidated gravel water-bearing formation; depths 60 to 140 feet.	None.
Falls City	Seven drilled wells in Missouri River Valley; depth 60 to 65 feet; water-bearing formation unconsolidated sand and gravel. (For emergency use—4 drilled wells in Nemaha River Valley, depth approximately 40 feet water-bearing formation unconsolidated sand and gravel.)	Missouri Valley well supply only—aeration, lime softening, coagulation with alum, rapid sand filtration, chlorine disinfection.
Plattsmouth	Twelve drilled wells in Missouri River Valley all in unconsolidated sand and gravel water-bearing formation; depths 50 to 90 feet.	Iron and manganese removal with aeration, primary chlorination, ph control with lime, rapid sand filtration and disinfection with chlorine.
Holdrege	Seven drilled wells, all in unconsolidated sand and gravel water-bearing formation; depths 189 to 198 feet.	None.
Crete	Three drilled wells—all in unconsolidated sand and gravel water-bearing formation; depths 80 to 90 feet.	Do.
Wayne	Three drilled wells; all in unconsolidated sand and gravel water-bearing formation; depths 128 to 140 feet.	Do.
Seward	Six drilled wells; all in unconsolidated sand and gravel water-bearing formation; depth 110 to 120 feet.	Do.
Wahoo	Three drilled wells—all in unconsolidated sand and gravel water-bearing formation; depths 80 to 85 feet.	Do.
Schuyler	Four drilled wells—all in unconsolidated sand and gravel water-bearing formation; depths 33 to 70 feet.	Do.
Atwood, Kansas	Two drilled wells—both in unconsolidated sand and gravel water-bearing formation; depth approximately 50 feet.	Chlorination.

TABLE 5.—*Summary of the source and treatment of the water supplies of the study cities*—Continued

City	Source of water supply	Treatment of water supply
Colby, Kansas	One dug well, 5 drilled wells; all in same unconsolidated sand and gravel water-bearing formation; depths: No. 1, No. 2, No. 3 and No. 4 (dug) approximately 155 feet; No. 5 and No. 6 approximately 230 feet.	Do.
Goodland, Kansas	Six drilled wells, all in same sandstone and unconsolidated sand and gravel water-bearing formation; depths 265 to 300 feet.	Do.
St. Francis Kansas.	Seven drilled wells, all in same Republican River Valley alluvium water-bearing formation; depths 25 to 28 feet.	Do.

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[1] Also a number of small diameter shallow (20 to 30 foot depth) wells in immediate vicinity of above-mentioned wells were used as supplementary source until abandonment in 1946.

THE FLUORIDATION OF DRINKING WATER AND THE CONTROL OF DENTAL CARIES

Reduction In Dental Caries By Fluoridated Drinking Water

Since January 25, 1945, the community drinking water of Grand Rapids, Mich., has been continuously fluoridated at a level of 1.00 p.p.m. fluoride. By means of yearly dental examinations of children aged 4 to 16 years who were residents of Grand Rapids after that date, the cariostatic effect of fluoridated drinking water has been conclusively demonstrated. The reduction in dental caries, which amounts to upwards of 60 percent, simulates the caries reducing effect of drinking waters containing a comparable level of fluoride as a natural constituent.

Whereas caries inhibition appears to be a function of the duration of fluoride exposure prior to tooth eruption and the risk of the caries attack, fluoridated drinking water has caries inhibitory effects also on teeth exposed posteruptively to this water.

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STUDIES ON MASS CONTROL OF DENTAL CARIES THROUGH FLUORIDATION OF THE PUBLIC WATER SUPPLY

By H. Trendley Dean, Francis A. Arnold, Jr., Philip Jay, and John W. Knutson

Numerous epidemiological studies (*1*) conducted in widely separated parts of the world clearly demonstrate that the use of fluoride drinking water during the formative period of the teeth is associated with a 60- to 65-percent reduction in dental caries experience. This inverse relationship between dental caries prevalence and fluorides in drinking water approaches its maximum at a fluoride (F) concentration of 1.0 to 1.5 p.p.m., a concentration which Dean (*2*) established as the minimal threshold for mottled enamel or endemic fluorosis. These findings led to the proposal that optimum amounts of fluorides be added to the drinking water supply as a partial caries-control measure. The proposal engendered extensive field and laboratory studies on the physiological effects of fluoride ingestion (*1*). The results of these studies indicated that not only was 1.0 p.p.m. in the drinking water an optimal concentration for caries control but well within the limits of safety.

In 1945, three studies to determine the caries prophylactic value of artificially fluoridated drinking water were started in the United States and Canada. A number of additional study projects have been initiated in the United States since that time. One of the sudies started in 1945, that in Grand Rapids, Mich., serves as the basis for this preliminary report.

MATERIAL AND METHODS

In order to afford a direct control on the observations during fluoridation of the drinking water supply at Grand Rapids, Mich., a control city, Muskegon, Mich., whose source of drinking water supply and geographical and climatological characteristics were similar to those of Grand Rapids, was selected. In addition, data were collected for direct comparisons with dental caries rates in Aurora, Ill., where the naturally occurring fluoride concentration of the public water supply is 1.2 p.p.m. of F. The base-line information collected consisted of detailed dental examinations of virtually the entire school population of Grand Rapids, Muskegon, and Aurora, those in continuous residence being selected for comparison in this report. The 1949 samples used for comparison in this report were taken from the kindergarten, and first, fourth, eighth, and eleventh grades. Examination of children in grade groups rather than in individual age groups has proven expedient in scheduling and conducting examinations in schools. These particular grade groups were selected to provide data principally regarding children 5, 6, 9, 13, and 16 years of age.

Fluoridation of the Grand Rapids water supply was started January 25, 1945. Sodium fluoride, over 90 percent pure, commercial grade, was used as the source of fluorine. Automatic feeders were employed to control the amount of sodium fluoride fed into the water supply, and daily tests were made at different points in the distribution system to assure maintenance of a uniform concentration of 1.0 p.p.m. The mechanics of adding the fluoride to the water supply is relatively simple and the desired concentration was maintained (*3*). Examination of samples of children in Grand Rapids and Muskegon have been made yearly and will continue to be made for the duration of the study. In making the dental examinations, mouth mirror and explorer were used under good lighting conditions. The examinations were conducted in the school buildings and the findings recorded in a precoded system for direct transfer to punch cards so that the processing of the data could be handled with mechanical devices. The number of children examined in 1944–45 and in 1949 and their distribution by age are shown in table 1.

FINDINGS

Deciduous Teeth. The dental caries experience in the deciduous teeth, expressed as def (decayed, extraction indicated, or filled) teeth per child, is shown in table 2. In the 1944–45 examinations of Grand Rapids children, 323 4-year-olds had 4.2 def teeth per child; 1,633 5-year-olds, 5.4 per child; 1,789 6-year-olds, 6.4 per child; and 1,806 7-year-olds, 6.3 per child. In 1949, for Grand Rapids children, 75 4-year-olds had 2.7 def teeth per child; 777 5-year-olds, 3.3; 697 6-year-olds, 4.6; and 54 7-year-olds, 4.8. It is likely that the 1949 4-year-olds were somewhat older than the 1944–45 4-year-olds since they were all enrolled in kindergarten, whereas 1944–45 4-year-olds included nursery school or pre-kindergarten children. The reduction in the 1949 Grand Rapids rates by comparison with those for 1944–45 was [illegible].7, 38.9, 28.1, and 23.8 percent, respectively. The Muskegon data (table 2) show for the 1944–45 base line 402 5-year-olds with 6.8 def teeth per child, and for 462 6-year-olds, 7.2. The 1949 examinations of 340 5-year-old children and 393 6-year-old children show 5.6 and 6.0 def teeth per

Table 1.—*Distribution of children examined at Grand Rapids, Muskegon, and Aurora, according to age, continuity of residence, and year of examination*

Age	Grand Rapids, Mich.				Aurora, Ill.		Muskegon, Mich.			
	1944–45		1949–50		1945–46		1944–45		1949–50	
	Total	Contin. res.	Total	Contin. res.	Total	Contin. res.	Total	Contin. res.	Total	Contin. res.
4	396	323	101	75	40	30	23	20	64	51
5	2,163	1,633	1,050	777	573	407	570	402	462	340
6	2,425	1,789	994	697	721	473	760	462	534	393
7	2,481	1,806	94	54	774	516	679	408	50	30
8	2,355	1,647	198	155	723	469	678	376	22	12
9	2,371	1,639	686	519	610	368	660	357	269	197
10	2,323	1,626	187	125	645	397	682	359	81	52
11	2,309	1,556	188	140	614	383	603	293	198	146
12	2,483	1,685	190	130	645	401	623	328	66	28
13	2,498	1,668	779	574	661	401	662	377	333	214
14	2,658	1,690	218	153	801	433	717	369	119	66
15	2,431	1,511	111	64	872	467	648	292	76	34
16	1,721	1,107	306	209	633	371	481	248	219	132
Total	28,614	19,680	5,102	3,672	8,312	5,116	7,786	4,291	2,493	1,695

child, respectively, a percentage reduction from the Muskegon 1944–45 base line of 17.7 and 16.7 percent, respectively. It should be noted, however, that the 1944–45 rates for these age groups were somewhat higher than the 1944–45 baseline rates at Grand Rapids.

The school population of Aurora was examined in the fall of 1945 for the purpose of developing a caries experience expectancy curve. The Aurora data (table 2) show a strikingly lower caries experience when compared with the Grand Rapids base-line data. As previously noted, the 1949 examinations at Grand Rapids showed reductions of 35.7, 38.9, 28.1, and 23.8 when compared with the 1944–45 rates. If the rates observed at Aurora are compared with the 1944–45 Grand Rapids data, reductions of 50.0, 48.2, 46.9, and 44.4 percent would be expected. Attention is called, however, to the inadequacy of the sample in the 4- and 7-year-olds in the 1949 Grand Rapids sample and to the fact that none of the 6-year-olds had used fluoride water continuously since birth.

Permanent Teeth. Table 3 shows the DMF (decayed, missing, or filled teeth) rates for the permanent teeth of children aged 5 through 16 who were continuous residents. In 1944–45 at Grand Rapids, there were examined 1,789 6-year-old children, 1,806 7-year-olds, 1,647 8-year-olds, 1,639 9-year-olds; these specific age groups showed DMF rates of 0.78, 1.89, 2.94, and 3.90, respectively. The 1949 Grand Rapids examination of 697, 54, 155, and 519 children in these respective age groups showed 0.38, 0.76, 2.16, and 2.48 DMF teeth per child or a reduction of 51.3, 59.8, 26.5, and 36.4 percent, respectively. Attention is called to the size of the samples of 7- and 8-year-olds and the selection of sample by school grade rather than by age.

At Muskegon, the 1944–45 examinations included 462 6-year-olds; 408 7-year-olds, 376 8-year-olds, and 357 9-year-olds, with a DMF experience of 0.81, 1.99, 2.81, and 3.81, respectively. The 1949 examination embraced 393 6-year-olds, 30 7-year-olds, 12 8-year-olds, and 197 9-year-olds, with a DMF rate of 0.63, 1.43, 2.58, and 3.88, respectively. As is apparent from the numbers shown, only the 6- and 9-year-olds warrant comparison. The 6-year-olds in 1949 showed a 22.2 percent reduction from the 1944–45 rate; the 9-year-olds, a 1.8 percent increase.

If one compares the rates of 5-, 6-, and 7-year-olds at Aurora in 1945 with the present Grand Rapids rates for children in these age groups, it is noted that the DMF experience is quite similar. When these rates are compared with the 1944–45 Grand Rapids rates, the percentage reductions also tend to be alike. How-

TABLE 2.—*Dental caries experience, deciduous teeth, observed among 27,308 children, age 4–13, of Grand Rapids, Muskegon, and Aurora, expressed as def teeth per child with percentage reductions observed (continuous residents)*

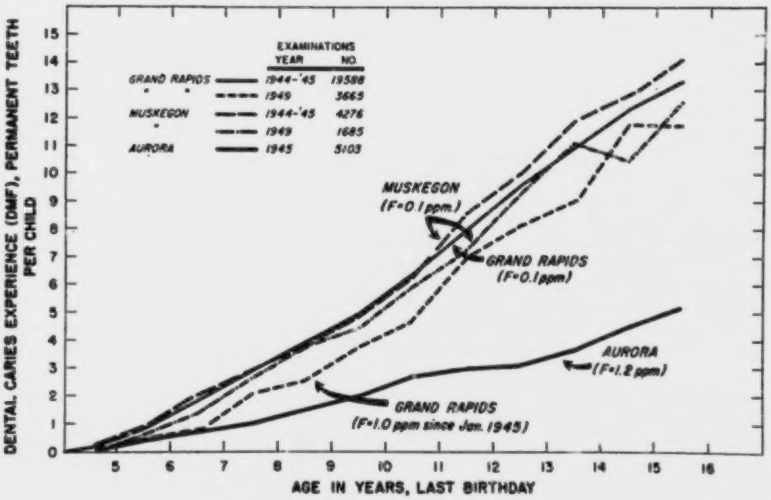
Age	Grand Rapids, Mich.			Aurora, Ill.		Muskegon, Mich.		
	Examinations made		Percentage reduction	Examinations 1945–46	Percent less than G. R. 1944–45	Examinations made		Percentage less
	1944–45	1949–50				1944–45	1949–50	
4	4.2	2.7	35.7	2.1	50.0	------	4.4	------
5	5.4	3.3	38.9	2.8	48.2	6.8	5.6	17.7
6	6.4	4.6	28.1	3.4	46.9	7.2	6.0	16.7
7	6.3	4.8	23.8	3.5	44.4	6.7	------	------
8	5.8	4.7	19.0	3.6	37.9	6.1	------	------
9	4.6	4.4	4.3	3.0	34.8	4.9	4.5	8.2
10	2.8	2.9	−3.6	2.3	17.9	3.1	2.8	9.7
11	1.3	1.2	7.7	1.2	7.7	1.3	1.2	7.7
12	.5	.4	20.0	.4	20.0	------	------	------
13	.2	.1	50.0	.1	50.0	------	------	------

TABLE 3.—*Dental caries experience, permanent teeth, observed among 33,955 children, age 5–16, of Grand Rapids, Muskegon, and Aurora, expressed as DMF teeth per child with percentage reductions observed (continuous residents)*

Age	Grand Rapids, Mich.			Aurora, Ill.		Muskegon, Mich.		
	Examinations made		Percentage reduction	Examinations 1945–46	Percent less than G. R. 1944–45	Examinations made		Percentage change
	1944–45	1949–50				1944–45	1949–50	
5	0.11	0.03	72.7	0.06	45.5	0.06	0.14	+133.3
6	.78	.38	51.3	.28	64.1	.81	.63	−22.2
7	1.89	.76	59.8	.70	63.0	1.99	1.43	−28.2
8	2.94	2.16	26.5	1.04	64.6	2.81	2.58	−8.2
9	3.90	2.48	36.4	1.52	61.0	3.81	3.88	+1.8
10	4.92	3.56	27.7	2.02	59.0	4.91	4.44	−9.6
11	6.41	4.69	26.8	2.67	58.4	6.32	5.93	−6.2
12	8.07	7.02	13.0	2.95	63.5	8.66	7.21	−16.8
13	9.73	8.11	16.7	3.09	68.3	9.98	9.52	−4.6
14	10.94	8.90	18.6	3.64	66.7	12.00	11.08	−7.7
15	12.48	11.80	5.5	4.54	63.6	12.86	10.32	−19.8
16	13.50	11.83	12.4	5.19	61.6	14.07	12.51	−11.1

Amount of dental caries experience (DMF), permanent teeth in Grand Rapids, Muskegon, and Aurora school children

(Continuous residents)



ever, a very limited number of erupted permanent teeth in 5-year-old children and a small sample of children in the 7-year-old group for Grand Rapids in 1949 are the basis for these rates.

The chart summarizes graphically the tabular data given in table 3. The lines show the dental caries experience for the permanent teeth of children aged 5 through 16. The upper solid black line shows the dental caries prevalence recorded for the 19,680 continuous residents in the Grand Rapids 1944–45 base-line study. The long dash line shows the prevalence rate, 1944–45, at Muskegon, the control city. As may be seen, these base-line prevalence rates are almost identical until about 12 years of age when Muskegon's rates are slightly higher than Grand Rapids. The lower solid black line is the Aurora curve, based upon 5,116 examinations made in 1945.

An examination of the 1949 prevalance rates computed for Grand Rapids and Muskegon shows that in the latter city the curve roughly follows the 1944–45 base line. Note that for the Grand Rapids 1949 trend line the points for the 5-, 6- and 7-year-old children fall on the Aurora line, but for older children, aged 8 to 16 years, the Grand Rapids 1949 trend line is appreciably above that of Aurora. The slope of the 1949 Grand Rapids line beginning with the 8-year-olds is very similar to that of the Grand Rapids 1944–45 line. This finding suggests that Grand Rapids children, ages 8 to 16, are accumulating new carious permanent teeth at the same rate in 1949 as children in this range were accumulating them in 1944–45. In other words, the 5-, 6-, and 7-year-olds of Grand Rapids in 1949 are on the Aurora expectancy curve. The very limited number of permanent teeth in 5-year-olds and the small sample of 7-year-olds, however, make caution mandatory in interpretation of results. On the other hand, although the permanent teeth in 6-year-old children have a very limited exposure time to dental caries attack, the large sample, 697, examined in 1949 at Grand Rapids, justifies comparison with the 473 Aurora children in the same age group. The 1950 examinations which will include additional grades in the lower age groups should reveal highly important data.

SUMMARY

Fluoridation of the Grand Rapids public water supply began in January 1945. Analysis of the 1949 dental examinations at Grand Rapids shows a reduced amount of dental caries experience when compared with the prefluoridation rates of 1944–45. The findings indicate that the reduction is most pronounced in the younger age groups whose dentition was largely calcified following the addition of one part per million of fluoride (F) to the previously fluoride-free public water supply. Sufficient time has not elapsed to evaluate water fluoridation in the older age groups.

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EFFECT OF FLUORIDATED PUBLIC WATER SUPPLIES ON DENTAL CARIES PREVALENCE. Seventh Year of Grand Rapids-Muskegon Study

By Francis A. Arnold, Jr., T. Trendley Dean, and John W. Knutson

Subsequent to the epidemiological and experimental findings that the use of fluoride waters reduced the prevalence of dental caries, a number of studies were undertaken to evaluate the effects of adjusting the fluoride levels of public water supplies to an optimum concentration, about 1 p.p.m. of fluoride (F). Preliminary and annual reports from these study areas have appeared in the literature from time to time during the past 5 or 6 years (*1–9*). Essentially, the findings of all these studies are in accord although each has its individual characteristics from a procedural standpoint.

The present paper reports in more detail than a preliminary report (*1*) the methodology and results of one of these studies, namely, the Grand Rapids-Muskegon study, and includes the results obtained after 6½ years of fluoridation of the Grand Rapids water supply.

SELECTION OF STUDY AREA

Because much of the background data regarding the effects of fluoride waters on dental caries was obtained in the north central part of the United States, this general area was selected for the fluoridation project. The choice was further influenced by the fact that (*a*) a considerable amount of data had been obtained on children who had used domestic water supplies for which Lake Michigan was the source, and (*b*) all evidence suggested that this general area had a high dental caries experience rate (*10*). Following a careful survey of a number of communities in this area, and with due consideration of the numerous details which influence a project of this sort, the city of Grand Rapids, Mich., was selected for the primary study area. The city of Muskegon, Mich., which has the same source of water and similar treatment procedures as Grand Rapids, was chosen as a control area. To establish what might be termed an "expectancy curve" for this study, a natural fluoride area, the city of Aurora, Ill., was selected. The Aurora water supply contains 1.2 p.p.m. F and has a reliable history of constancy back to 1895 (*11*).

BASE-LINE EXAMINATIONS

Dental examinations were started in Grand Rapids in September 1944. Four trained dental officers performed the examinations in selected schoolrooms, using mouth mirrors and explorers, and standard type examining lights. The examining equipment throughout the study has been a double-end SSW No. 3 explorer and

TABLE 1.—*Distribution of continuous resident children examined in Grand Rapids and Muskegon, Mich., and in Aurora, Ill., according to age and year of examination*

Age last birthday	1945 Aurora, Ill.	Basic examinations 1944–45	1945	1946	1947	1948	1949	1950	1951
		Grand Rapids, Mich.							
4	30	323	540	300	168	137	75	117	168
5	407	1, 633	1, 714	831	886	842	777	720	853
6	473	1, 789	1, 186	628	663	736	697	748	750
7	516	1, 806	149	82	69	55	54	438	423
8	469	1, 647	15	216	135	138	155	501	470
9	368	1, 639	--------	525	465	484	519	520	582
10	397	1, 626	--------	109	108	111	125	131	141
11	383	1, 556	--------	17	18	22	140	130	151
12	401	1, 685	174	85	38	60	130	200	176
13	401	1, 668	953	547	625	600	574	530	497
14	433	1, 690	273	173	196	152	153	130	128
15	467	1, 511	80	53	80	64	64	58	53
16	371	1, 107	4	3	233	245	209	177	198
		Muskegon, Mich.							
4	--------	20	--------	43	18	26	51	41	63
5	--------	402	--------	321	348	422	340	359	351
6	--------	462	--------	339	312	305	393	310	294
7	--------	408	--------	36	42	36	30	274	223
8	--------	376	--------	18	13	10	12	190	275
9	--------	357	--------	213	215	199	197	227	277
10	--------	359	--------	62	57	52	52	51	62
11	--------	293	--------	12	10	14	146	141	139
12	--------	328	--------	21	19	11	28	43	48
13	--------	377	--------	197	207	208	214	173	225
14	--------	369	--------	77	50	79	66	63	59
15	--------	292	--------	18	44	41	34	35	21
16	--------	248	--------	1	199	205	132	146	155

standard plain mirror. The source of light has been a standard E.E.N.T. examination lamp. Bite-wing X-ray examinations were made of a representative sample of children examined by the different examiners to evaluate, in part, the "examiner error." The result of this evaluation will be the subject of another report. A complete oral examination was made and a residence history obtained, which, for the younger children, was verified by a questionnaire signed by parent or guardian. Only the principal dental findings will be considered here.

The examiner dictated his findings in code to recording scribes. The code used for recording dental conditions is similar to one described previously (*12*). It is designed to give a complete description of the health status of all teeth and each surface of individual teeth, both separately and collectively. A short "indoctrination" period was used to acquaint the examiners and scribes with the code and to establish consistency among examiners regarding the subjective assessment features of diagnosis. Virtually all pupils of the school systems of the selected cities were examined, including nursery schools and junior college students where available.

SUBSEQUENT ANNUAL EXAMINATIONS

Following the original "prefluoridation" examinations, annual dental examinations have been made in Grand Rapids and Muskegon. These examinations are being conducted in the same manner as were the original ones. There have been changes in dental examiners with the exception of one officer who has participated in each series of examinations. Each new examiner has been calibrated against this one officer to standardize diagnostic criteria.

These annual examinations are made at the same time each year (October and November) and are performed on selected samples of the school children. This annual study sample was selected after careful review of census data and consultation with city planning department officials. On the basis of available information, the 31 school districts of Grand Rapids were classified on a socioeconomic basis. From the 79 schools in these districts, 25 representative schools were selected, and the examiners were assigned schools on a basis of equal-sized samples of comparable population groups. In Muskegon, the annual examinations have been conducted in almost all schools, excluding only a few small schools on the periphery of the city where many students are from rural areas. The examiners work in the same schools each year. Each new examiner is assigned the schools which had previously been assigned to the examiner whom he replaced.

Selected age groups of children are examined within each of the schools.

Table 2.—*Dental caries experience in deciduous and permanent teeth of continuous resident children of Aurora, Ill., as observed in the 1945–46 examination period*

Age last birthday	Deciduous teeth		Permanent teeth				
	Number filled	Total def [1]	Decayed	Missing [2]	Filled	Total DMF [3]	Percent of children with DMF teeth
	Number of teeth per child						
4	0. 10	2. 07	0	0	0	0	------
5	. 25	2. 79	. 059	0	0	. 059	2. 7
6	. 38	3. 36	. 263	. 002	. 015	. 280	15. 2
7	. 44	3. 51	. 661	. 009	. 035	. 705	33. 9
8	. 54	3. 60	. 917	. 014	. 111	1. 042	44. 8
9	. 49	2. 98	1. 185	. 063	. 274	1. 522	55. 2
10	. 27	2. 28	1. 426	. 089	. 505	2. 020	66. 5
11	. 15	1. 18	1. 755	. 227	. 688	2. 670	72. 3
12	. 05	. 43	1. 774	. 219	. 958	2. 951	73. 1
13	. 01	. 13	1. 658	. 236	1. 195	3. 089	73. 3
14	------	------	1. 750	. 305	1. 584	3. 639	78. 3
15	------	------	1. 989	. 501	2. 047	4. 537	83. 1
16	------	------	1. 941	. 533	2. 712	5. 186	85. 4

[1] Decayed, extraction indicated, or filled deciduous teeth.

[2] Includes teeth listed as "remaining roots" and teeth destroyed beyond any possible repair.

[3] Decayed, missing, or filled permanent teeth. Each tooth is counted only once. A tooth which shows a filled surface is considered a filled tooth regardless of whether or not it has additional carious areas.

Selection is made on the basis of school grade or class, using all children present in a class or grade of a school. Choosing examinees by grade in this manner will, in some instances, not give well-distributed specific age groupings. For example, an eighth-grade group will give a well-distributed 13-year-old age group, and will include the older 12-year-olds and younger 14-year-olds when specific age groups are based on age at last birthday. The number of grades included in the 1951 examinations give well-distributed age groupings through the 13-year-old groups.

Each child in a selected grade or class is examined regardless of his residence history. The examiner has no knowledge at the time of examination whether or not the individual examinee is a "continuous resident" of the city in which the examinations are being held.

The following table shows the grades, or selected class groups from these grades, which were included for each annual (fall) examination in Grand Rapids and Muskegon subsequent to the original or basic examinations:

Year	*Grade or class*
1945......	Kindergarten, 1, 8
1946......	Kindergarten, 1, 4, 8
1947......	Kindergarten, 1, 4, 8, 11
1948......	Kindergarten, 1, 4, 8, 11
1949......	Kindergarten, 1, 4, 6, 8, 11
1950......	Kindergarten, 1, 2, 3, 4, 6, 8, 11
1951......	Kindergarten, 1, 2, 3, 4, 6, 8, 11

In addition to the routine dental examinations in these two cities, special studies, including bacteriological and chemical studies of the saliva, are being made on selected groups of children. The results of these studies will be reported at a later date.

Fluoridation

The addition of sodium fluoride (NaF) to the Grand Rapids water supply started in January 1945. Since that time, the people of this city have been ingesting a water with a fluoride content of 1 p.p.m. F. Daily analyses show that this concentration has been maintained within a 0.2 p.p.m. range (*13*). No significant mechanical difficulties of any consequence have been encountered. Also, in the course of 7 years of fluoridation, no established complaints of any ill effects have been encountered from the numerous manufacturing and processing concerns, nor from the population involved.

The water supply at Muskegon remained unchanged until July 1951, when this city started adding fluorides to its water supply.

Results

Base-line data were obtained on the oral health status of 31,007 Grand Rapids residents aged 2–24 years, 8,304 Muskegon residents aged 4–25 years, and 8,811 Aurora, Ill., residents aged 4–20 years. The present report includes only the findings in regard to dental caries in "continuous resident" children aged 4–16 years. This selection excludes the results on children of these ages who have lived outside their respective communities for more than 3 months in any one calendar year. Table 1 shows the number of continuous resident children of these ages examined each year and their distribution by age.

For purposes of comparison, the results of oral examinations of Aurora school children are shown in table 2. As mentioned previously, the results at Aurora represent the caries experience in children who have used water containing 1.2 p.p.m. F throughout life. It is this same caries experience that one might expect to find in the teeth of Grand Rapids children after fluoridation has been in progress a comparable period of time in respect to the specific age group under comparison.

It should be noted that the dental examiners in this study obtained similar results to those findings previously re-

TABLE 3.—*Dental caries findings, deciduous teeth, in Grand Rapids and Muskegon, Mich., school children, 4–13 years of age, according to year of examination*

Age last birthday	Number of def[1] deciduous teeth per child							
	Basic examinations 1944–45	1945	1946	1947	1948	1949	1950	1951
	Grand Rapids, Mich.							
4	4. 186	5. 398	3. 427	3. 190	3. 022	2. 747	2. 462	2. 131
5	5. 369	6. 151	5. 083	3. 893	4. 027	3. 273	2. 501	2. 273
6	6. 431	6. 979	5. 725	5. 379	4. 784	4. 590	3. 730	2. 977
7	6. 293	7. 658	6. 110	5. 841	5. 200	4. 833	4. 715	4. 031
8	5. 782	8. 000	5. 097	5. 074	4. 877	4. 748	4. 908	4. 123
9	4. 591	--------	4. 446	4. 110	4. 428	4. 410	4. 229	3. 856
10	2. 837	--------	2. 835	3. 157	3. 063	2. 856	2. 359	2. 426
11	1. 345	--------	2. 118	2. 778	1. 773	1. 193	1. 162	1. 351
12	. 473	. 276	. 129	. 105	. 250	. 354	. 245	. 295
13	. 176	. 130	. 139	. 136	. 170	. 103	. 147	. 117
	Muskegon, Mich.							
4	5. 050	(2)	3. 442	4. 667	4. 385	4. 412	5. 317	4. 460
5	6. 820	--------	5. 860	5. 052	5. 552	5. 556	5. 349	5. 248
6	7. 167	--------	6. 239	6. 179	6. 056	5. 992	6. 019	5. 667
7	6. 663	--------	6. 833	5. 952	6. 917	6. 333	5. 825	5. 771
8	6. 061	--------	4. 833	3. 846	4. 800	6. 083	5. 063	5. 320
9	4. 885	--------	4. 315	4. 344	4. 714	4. 482	4. 088	4. 173
10	3. 084	--------	3. 145	3. 667	2. 788	2. 769	3. 490	2. 855
11	1. 328	--------	1. 667	2. 900	. 643	1. 212	1. 085	1. 460
12	. 422	--------	. 143	. 368	. 636	. 679	. 605	. 312
13	. 234	--------	. 289	. 174	. 106	. 112	. 127	. 147

[1] Decayed, extraction indicated, or filled deciduous teeth. A decayed and filled tooth is counted only as a filled tooth.

[2] The 1944–45 basic examinations in Muskegon were not done until late spring of 1945; therefore, no repeat examinations were made in the fall of 1945.

ported in the basic epidemiological studies for 12–14-year-old children of Aurora, Ill. (*11*).

The amount of dental caries experience observed at each examination in the deciduous and permanent teeth in both the study and control areas is shown in tables 3 and 4. There has been a reduction in the amount of dental caries observed in the Grand Rapids children subsequent to fluoridation of their water supply. For example, comparison of the 1951 and 1944–45 findings on permanent teeth shows this reduction to vary from 66.6 percent in the 6-year-old children to 18.1 percent in the 16-year-olds (table 4 and fig. 2). A similar comparison of results at Muskegon shows the percentage reduction to range from 1.5 percent in 6-year-olds to a high of 15.5 percent in the 11-year-olds. In Muskegon, the number of examinations was smaller, especially in the older age group. The 5-year-old group of 1951 is older by an average of 4 to 5 months than the same age group of 1944–45. Preschool children were examined in 1944–45, but in 1951 only children old enough for entrance to kindergarten were examined. It should be noted that the percentage reductions observed at Muskegon do not fit a consistent pattern as do those at Grand Rapids and may, in part, represent sampling error.

In establishing DMF rates, two portions of this rate are derived from observations based almost entirely on objective assessment, namely, the number of missing teeth and the number of filled teeth. Table 5 shows a comparison of the number of missing and filled permanent teeth for the study and control areas. There has been a reduction in the average number of missing teeth observed in Grand Rapids children up to and including the 13-year-old age group. This situation did not prevail in Muskegon. The findings in regard to the average number of filled teeth in these two areas would not explain the reduction in missing teeth. There has been little change in the average number of filled teeth in Grand Rapids whereas in Muskegon this average shows some indication of having increased slightly in the younger age groups.

DISCUSSION

The 1951 results in this report represent the findings of the seventh year of a study on the effect of fluoridation of public water supplies on dental caries prevalence. The results in Grand Rapids for 1951 represent findings after 6½ years

TABLE 4.—*Dental caries findings, permanent teeth, in Grand Rapids and Muskegon, Mich., school children, 5–16 years of age, according to year of examination*

Age last birthday	Number of DMF [1] permanent teeth per child							
	Basic examinations 1944–45	1945	1946	1947	1948	1949	1950	1951
	Grand Rapids, Mich.							
5	0. 109	0. 082	0. 049	0. 038	0. 038	0. 031	0. 028	0. 048
6	. 775	. 558	. 234	. 367	. 262	. 380	. 261	. 259
7	1. 886	1. 718	1. 110	1. 087	1. 036	. 759	1. 034	. 844
8	2. 945	3. 267	2. 542	2. 615	2. 304	2. 155	1. 766	1. 577
9	3. 898	--------	2. 981	3. 116	2. 671	2. 478	2. 383	2. 040
10	4. 921	--------	3. 697	3. 556	3. 514	3. 560	3. 168	2. 929
11	6. 409	--------	4. 235	3. 556	4. 318	4. 686	4. 362	3. 669
12	8. 073	9. 529	7. 624	7. 026	8. 317	7. 015	7. 100	5. 886
13	9. 734	10. 759	8. 920	8. 469	8. 338	8. 111	7. 206	6. 600
14	10. 945	11. 901	9. 410	9. 500	9. 414	8. 895	8. 546	8. 211
15	12. 482	12. 675	11. 264	11. 938	10. 609	11. 797	10. 121	8. 906
16	13. 499	13. 000	9. 333	12. 472	13. 502	11. 833	11. 350	11. 061
	Muskegon, Mich.							
5	. 065	(2)	. 044	. 069	. 062	. 135	. 086	. 117
6	. 811	--------	. 481	. 657	. 790	. 631	. 748	. 799
7	1. 988	--------	1. 331	1. 048	2. 194	1. 433	2. 011	1. 879
8	2. 810	--------	2. 833	2. 154	3. 500	2. 583	2. 958	2. 629
9	3. 808	--------	3. 291	3. 535	3. 578	3. 883	3. 894	3. 516
10	4. 906	--------	4. 274	3. 596	4. 865	4. 442	4. 529	4. 323
11	6. 318	--------	4. 250	4. 700	4. 714	5. 932	5. 667	5. 338
12	8. 655	--------	8. 429	6. 789	7. 818	7. 214	6. 884	7. 708
13	9. 981	--------	9. 015	9. 227	10. 524	9. 523	9. 578	9. 364
14	11. 995	--------	11. 091	12. 000	12. 266	11. 076	12. 111	11. 356
15	12. 862	--------	11. 167	12. 886	12. 659	10. 324	10. 943	12. 381
16	14. 068	--------	19. 000	12. 769	14. 307	12. 508	13. 911	13. 161

[1] Decayed, missing, or filled permanent teeth. A decayed and filled tooth is counted only as a filled tooth.

[2] The 1944–45 basic examinations in Muskegon were not done until the late spring of 1945; therefore, no repeat examinations were done in the fall of 1945.

of exposure to a fluoride water environment. The tabular data indicate a definite reduction in age specific caries rates in Grand Rapids children. As shown in figures 1 and 2 the observed caries rates for Grand Rapids have changed over the past 6 to 7 years and in the younger age groups are approaching the findings on continuous resident children of Aurora, Ill., in 1945–46.

It is of special interest to note the increment in dental caries between ages 6 and 9 years. In Grand Rapids for this age period in the basic examinations, this increment was 3.123 DMF teeth per child as compared to 1.781 DMF teeth per child in the 1951 examinations. For Muskegon children, the basic examination results indicate an incremental increase between the years of 6 to 9 of 2.997 DMF teeth as compared to 2.717 as observed in 1951. Thus, the increment of dental caries over this 3-year-age period has decreased in Grand Rapids by 1.34 DMF teeth while at Muskegon the decrease is only 0.28 DMF teeth.

Attention is called to the fact that DMF rates in this age group (6–9 years) reflect mainly the caries rates in first permanent molars. In other words, the results to date on Grand Rapids children indicate that their first permanent molar teeth are decaying at a rate which is only 57 percent of the 1944–45, or prefluoridation, rate. Also, considering that only the 6-year-old and part of the 7-year-old children were born into a fluoride en-

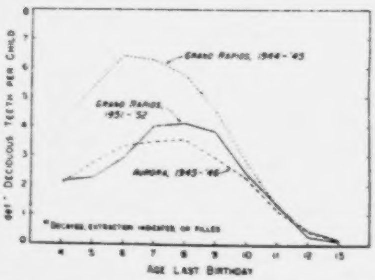

FIGURE 1.—*Comparison of dental caries experience in deciduous teeth of continuous resident school children in Grand Rapids, Mich., and Aurora, Ill.*

TABLE 5.—*Comparison of the number of filled or missing permanent teeth observed in Grand Rapids and Muskegon, Mich., children during the basic (1944–45) examination and the 1951 examination*

Age last birthday	Filled permanent teeth							
	Grand Rapids, Mich.				Muskegon, Mich.			
	Number per child		Percent of total DMF		Number per child		Percent of total DMF	
	1944–45	1951	1944–45	1951	1944–45	1951	1944–45	1951
5	0. 006	0. 006	5. 51	12. 50	0	0. 011	0	9. 40
6	. 072	. 068	9. 29	26. 26	. 063	. 109	7. 77	13. 64
7	. 338	. 317	17. 92	37. 56	. 312	. 404	15. 69	21. 50
8	. 822	. 834	27. 91	52. 89	. 719	. 927	25. 59	35. 26
9	1. 395	1. 220	35. 79	59. 80	1. 518	1. 690	39. 86	48. 07
10	1. 899	1. 270	38. 59	43. 86	2. 100	1. 613	42. 80	37. 31
11	2. 559	2. 437	39. 93	66. 42	2. 497	2. 237	39. 52	41. 91
12	3. 480	4. 239	43. 11	72. 02	3. 930	3. 000	45. 41	38. 92
13	4. 444	4. 525	45. 65	68. 56	4. 603	4. 907	46. 12	52. 40
14	5. 454	4. 602	49. 83	56. 05	5. 637	3. 797	46. 99	33. 44
15	6. 738	5. 604	53. 98	62. 92	6. 773	5. 476	52. 66	44. 23
16	8. 406	8. 263	62. 27	74. 70	8. 426	8. 916	59. 90	67. 75
	Missing permanent teeth							
5	0. 001	0			0	0		
6	. 001	0			. 004	0		
7	. 017	0			. 017	. 013		
8	. 044	. 013			. 037	. 044		
9	. 150	. 046			. 160	. 101		
10	. 281	. 163			. 241	339		
11	. 478	. 179			. 467	. 468		
12	. 645	. 460			. 741	. 854		
13	. 836	. 366			. 937	. 929		
14	1. 045	1. 195			1. 149	1. 085		
15	1. 239	. 849			1. 240	1. 857		
16	1. 501	1. 197			1. 500	1. 574		

vironment, this result is consistent with the findings for Aurora children.

It should be noted that the only known major change affecting the environment of these two groups of children over the period of study has been the fluoridation of the Grand Rapids water supply. There have been no concerted efforts to inaugurate any special caries control programs, such as topical fluoride programs, in either city since the study started. From an epidemiological standpoint the results of this study, together with those of other comparable studies, permit the conclusion that adjusting the fluoride content of public water supplies will result in a reduction of the incidence of dental caries in school children.

SUMMARY

The methodology and results after 7 years of the Grand Rapids-Muskegon study have been described. The 1951 results on continuous resident children after 6½ years of fluoridation of the Grand Rapids water supply indicate:

1. There has been a reduction in dental caries rates in permanent teeth of Grand Rapids children ranging from 66.6 percent in 6-year-old children to 18.1 percent in the 16-year age group. Similar results have been obtained regarding the deciduous teeth.

2. Similar reductions have not been observed in Muskegon where the water supply remained "fluoride-free" (<0.2 p.p.m. F) until the last 3 months of this study period.

3. This change in dental caries rates at Grand Rapids was also reflected in observations based on objective assessment, that is, a reduction in the number of missing teeth.

4. A comparison of the 1951 caries rates in Grand Rapids with those of Aurora, Ill., shows that insofar as can be determined to date the use of a fluoridated water gives the same beneficial effects as does the use of a natural fluoride water of similar concentration.

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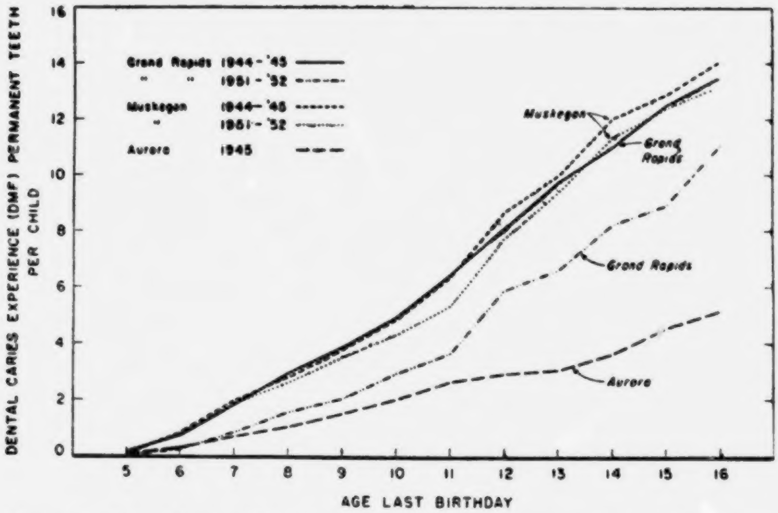

FIGURE 2.—*Comparison of dental caries experience in permanent teeth of continuous resident school children in Grand Rapids and Muskegon, Mich., and Aurora, Ill.*

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The authors also acknowledge the assistance given by the superintendents of education, principals, and teachers of their respective cities. And they acknowledge the cooperation of the health officials in the cities involved in this study.

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CLIMATE AND CONTROLLED FLUORIDATION

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The influence of climate in the determination of the optimum amount of fluoride introduced into community water supplies has been a matter of concern since fluoridation was first proposed as a caries control measure. Even before fluoridation became a public health reality, Arnold (*1*) pointed out that climate should influence water intake, and therefore total fluoride ingestion. In recent years may observers have suggested that temperature should be taken into consideration in establishing the fluoride level required to augment fluoride-deficient water supplies (*2–6*).

The influence of climate on water intake is now being observed in a limited fashion in the current approach to the fluoridation of community water supplies. For example, in Wisconsin the optimum fluoride concentration has been established at 1.2 p.p.m. whereas in the State of Florida it is set at 0.7 p.p.m. In Charlotte, N.C., the fluoride concentration is varied seasonally. In Charlotte, the water intake of children has been estimated by using the variation in soft drink consumption and bottled water sales volume as an index of total fluid intake. The fluoride level of the water supply is adjusted accordingly. Additional information about water intake is necessary, however, before the fluoride level of water supplies can be accurately adjusted to climatic conditions throughout the United States.

The early findings of the Smiths' in Arizona (*7, 8*), while not directly related to the influence of climate, indicate in retrospective analysis that in areas where high temperatures are experienced, an increased amount of fluorosis may be associated with less than the generally accepted fluoride concentration of 1.0 to 1.5 p.p.m. Recently, Dean (*5*) reported a similar relationship in two south Georgia communities. Children exposed to fluoride concentrations of 0.5 and 0.7 p.p.m. showed about the same prevalence of fluorosis which is associated with 1.0 p.p.m. or more in the Midwest.

Most of the studies (*9–12*) designed to measure the impact of climatic factors on water metabolism have been carried out on animals or on young adult males. The bulk of the investigations were conducted during World War II. They grew out of interest in the efficiency of military personnel in extreme climates.

In general these observations are in accord. Certain fundamental relationships seem clear. It has been amply demonstrated that excessive temperatures result in an increased body demand for fluid. Several other components of the total climatic environment may also influence water intake under certain conditions.

Whether these meteorological forces influence the water demand of infants and youth is subject to question. For a considerable portion of their tooth calcification period, their water consumption may be less closely related to natural climatic conditions than is the fluid intake of adults. Very little direct information exists regarding the water consumption of infants and children in relation to total climate. Certain limited observations are available, however.

Cooke (*13*) reports a study of six infants, made under controlled laboratory conditions, in which he observed a 71 percent increase in water consumption with an increase in temperature from 81° to 91° F. Cox (*14*) observed a similar influence in water consumption associated with an increase in mean daily temperature from 60° to 80° F. These findings are based on the observation of two children. Stadt (*6*) has made a limited study of the fluid intake of 1, 3 and 5 year old children for a one-week period during November and during July in Charlotte, N.C. He concluded that there was a 30 to 40 percent increase in fluid consumption during the summer season when the mean daily temperature increased from 50° to 78° F. These limited data suggest that the influence of temperature on water intake observed in the child are similar to those noted in the adult.

Comprehensive studies designed to measure the specific amount of fluid ingested by infants and children under ten years of age, exposed to natural environmental conditions, are difficult to conduct. This approach to the determination of the optimum concentration of fluoride in drinking water would involve an intricate reporting arrangement from a large number of families, broadly representative of the community, in areas with different climatic conditions, and covering a long period of time.

Dean (*5*) has suggested that a more practical solution to the problem might be to apply one or more simple biological tests that would measure indirectly the reaction of infants and youth to climatic influences on water intake. These tests might be the specific measurement of endemic fluorosis and dental caries experience in areas with variable climatic conditions. He has demonstrated a direct relationship between the fluoride concentration of community water supplies and the extent of endemic dental fluorosis, and an inverse relationship between the occurrence of that element and dental caries (*15, 16*). If varying degrees of fluorosis and dental caries experience are found to be associated with differences in climatic conditions, fluoride concentration of the communal water supply being the same, then from the indices of these two oral phenomena a rough comparison of water intake can be made. More important, should climate prove to be an influential factor, the concentration of fluoride used to supplement the community water supply can be adjusted in relation to the local climatic environment. In an effort to approach the problem this way, studies were designed to measure the influence of climate on water intake by the application of these two biological tests to children living in different geographical locations.

Ten communities from the classic "21 cities" investigation conducted some years ago by Dean (*17*) and his associates were selected to represent a base line area. These communities are all located in the Midwest, generally around Chicago, and they have fluoride concentrations in their water of around 1.0 p.p.m. These towns have served to a considerable extent as the basis for the determination that the optimum fluoride concentration is approximately 1.0 to 1.5 p.p.m. Their mean annual temperature is approximately 50° F.

The Southwest was selected as the first additional study area for several reasons (*18*). The South Central and Southwestern part of the State of Arizona, largely desert, is one of the hottest areas in the United States. Climatic influences on water intake, if any, should be readily apparent in a comparison of the biological indices of this area with those obtained in the base line communities. Also, fluoride-bearing waters are prevalent, and data on the fluoride content of municipal water supplies are abundant.

Six Arizona communities met the necessary requirements for such investigation. The towns had water supplies with adequate continuity, fluoride concentrations ranging around 1.0 p.p.m., and were of sufficient size to yield a sample of native-born children large enough to be significant. These six communities have a mean annual temperature of approximately 70° F.

The detailed data relative to the description of the water supplies, screening procedures, diagnostic methods and numbers of children examined are contained in the original reports of the two studies. The procedures followed were essentially the same in both groups of communities. Observations were limited to children of continuous residence. Diagnoses were made under similar conditions. The permanent teeth were assigned a mottled enamel classification as established by Dean (*15*). This tooth-unit classification was later transposed into the child-unit measurement for use in computing the fluorosis indices of the several communities. Notable differences between the two sets of observations are in the age groups involved and in the fact that different individuals made the diagnoses. Therefore, the data are subject to the error of examiner differences.

ENDEMIC DENTAL FLUOROSIS

The data on dental fluorosis observed by Dean and his associates in the ten Midwestern communities may be seen in table 1. The fluoride concentrations of these water supplies are presented in column 2; the prevalence of fluorosis in column 4. The percentage distribution of the signs of fluorosis is given in columns 5 through 10. It will be noted that moderate fluorosis was diagnosed in only 2 percent of the 3,249 children examined, and that there were no instances of severe fluorosis even when fluoride concentrations were well above 1.5 p.p.m. The community fluorosis indices in column 11 range from 0.25 associated with 0.4 p.p.m. of fluoride at Marion, Ohio, to 0.69 for Galesburg, Ill., which has 1.9 p.p.m. of fluoride in its water supply.

The fluorosis indices for these communities are presented graphically in figure 1. Dean (*15*) has suggested that a fluorosis index below 0.4 is of little or no public health concern. He considers indices ranging from 0.4 to 0.6 to be of borderline significance. For indices above 0.6, removal of excess fluoride is indicated. In figure 1 fluorosis indices have been plotted against fluoride concentra-

Table 1.—*Prevalence of fluorosis, percentage distribution of signs of fluorosis and community fluorosis indices in relation to fluoride concentrations of common water supplies continuously used by 3,249 children examined in ten Midwestern communities*

Community	Fluoride concentration	No. children examined	Per cent children affected	Percentage distribution of signs of fluorosis						Community fluorosis index
				Absent		Present				
				Normal	Question-able	Very mild	Mild	Moder-ate	Se-vere	
1	2	3	4	5	6	7	8	9	10	11
Marion, Ohio	0.4	263	6	57	37	5	1	–	–	0.25
Elgin, Ill.	0.5	403	4	61	35	4	1	–	–	0.22
Pueblo, Colo.	0.6	614	7	72	21	6	*	–	–	0.17
Kewanee, Ill.	0.9	123	12	53	35	10	2	–	–	0.31
Aurora, Ill.	1.2	633	15	53	32	14	1	–	–	0.32
East Moline, Ill.	1.2	152	32	37	32	30	2	–	–	0.49
Maywood, Ill.	1.2	171	33	39	28	29	4	–	–	0.51
Joliet, Ill.	1.3	447	25	41	34	22	3	–	–	0.46
Elmhurst, Ill.	1.8	170	40	28	32	30	9	1	–	0.67
Galesburg, Ill.	1.9	273	48	25	27	40	6	1	–	0.69

Age range: 12-14.

*Less than 0.5%.

Source: Fluorosis data from Dean, H. T. Epidemiological studies in the United States. In Dental caries anc fluorine. Lancaster, Pa., Science Press, 1946.

tion. It may be seen that the line representing the indices of the ten Midwestern communities crosses from the negative area into the borderline area at a fluoride concentration of approximately 1.1 p.p.m. It passes from the borderline area into the zone where fluoride removal is indicated between 1.6 and 1.7 p.p.m.

The fluorosis data obtained in the six Arizona communities are presented in table 2. The fluoride concentrations of these water supplies are presented in column 2, the prevalence of fluorosis in column 4. The distribution of fluorosis, percentagewise, is shown in columns 5 through 10. The occasional diagnosis of moderate and severe fluorosis in communities with fluoride concentrations of less than 1.0 p.p.m. is worthy of note. The community indices in column 11 range from 0.21 associated with 0.4 p.p.m. of fluoride at Yuma to 1.12 for Florence, which has 1.2 p.p.m. of fluoride in its water supply.

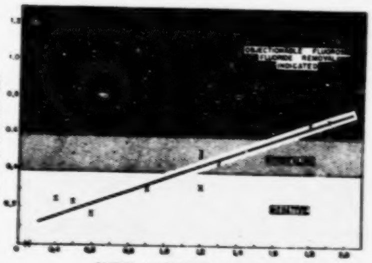
The community fluorosis indices for the Arizona area are presented graphically in figure 2. It will be noted that the indices line for these communities crosses from the negative area into the questionable zone at a fluoride concentration of approximately 0.6 p.p.m. The line moves from the borderline zone into that area where removal of excess fluoride is recommended at approximately 0.8 p.p.m.

The indices lines representing the two geographic areas are shown simultaneously in figure 3. At these concentrations the straight line progressive relationship between the fluoride concentration of the water supplies and the community fluorosis indices is apparent in both areas. The two lines merge at a fluoride concentration of approximately 0.4 p.p.m. At this concentration the discernible manifestations of fluorosis apparently are not evident enough to influence the community index. Beyond this point the lines diverge rapidly. Of extreme interest, however, is the fact that the line representing the Arizona communities accelerates at approximately twice the rate as does the line for the Midwestern area. These measurements of endemic fluorosis indicate that the children in the Arizona communities are ingesting approximately twice as much fluoride as the children in the Midwest when the concentration in the common water supplies is the same.

DENTAL CARIES

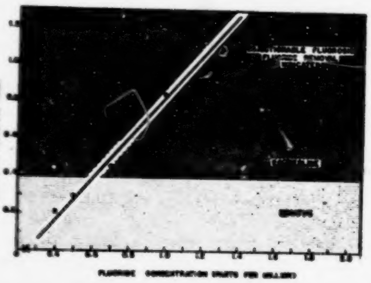
If this interpretation of the foregoing data is valid, the dental caries experience of the Arizona children consuming water with fluoride concentrations around 0.5 should be as low as that associated with a concentration of 1.0 p.p.m. in the Midwest.

The dental caries experience for the 12 to 14 year old children in the two groups of communities is presented in table 3. The DMF rate observed in the Midwestern communities with fluoride levels ranging about the optimum concentration of 1.0 p.p.m. has been boxed in column 5. As can be seen, it ranges from 258 DMF permanent teeth per 100 children in Maywood to 343 DMF teeth in Kewanee.



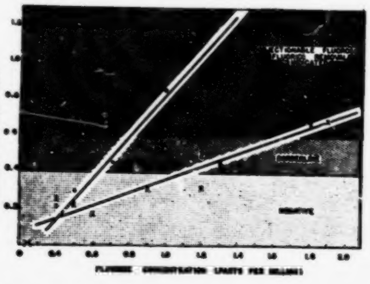
Trend line formula: $Y = 0.021 + 0.353X$.

Figure 1.—*Relationship between fluoride concentration of municipal waters and fluorosis index for communities with mean annual temperature of approximately 50° F. (Source: Table 1.)*



Trend line formula: $Y = -0.291 + 1.132X$.

Figure 2.—*Relationship between fluoride concentration of municipal waters and fluorosis index for communities with mean annual temperature of approximately 70° F. (Source: Table 2.)*



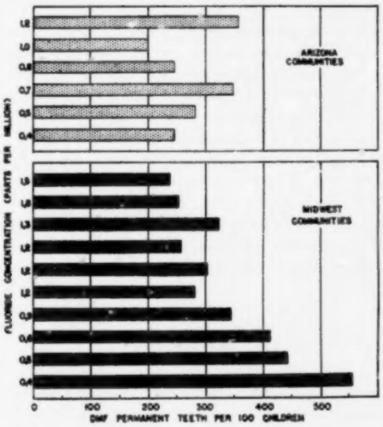
Mean annual temperatures: • Approximately 70°F. ($Y = 0.291 + 1.132X$). X Approximately 50°F. ($Y = 0.021 + 0.353X$).

Figure 3.—*Relationship between fluoride concentration of municipal waters and fluorosis index for communities with mean annual temperatures of approximately 50° F. and 70° F. (Source: Figures 1 and 2.)*

TABLE 2.—*Prevalence of fluorosis, percentage distribution of signs of fluorosis and community fluorosis indices in relation to fluoride concentrations of common water supplies continuously used by 726 children examined in six Arizona communities, 1951*

Community	Fluoride concentration	No. children examined	Per cent children affected	Percentage distribution of signs of fluorosis						Community fluorosis index
				Absent		Present				
				Normal	Questionable	Very mild	Mild	Moderate	Severe	
1	2	3	4	5	6	7	8	9	10	11
Yuma	0.4	82	4	65	32	2	1	—	–	0.21
Tempe	0.5	113	10	52	38	9	1	—	–	0.30
Tucson	0.7	316	17	38	45	12	3	2	–	0.46
Chandler	0.8	95	19	42	39	9	6	2	1	0.52
Casa Grande	1.0	50	48	14	38	30	18	—	–	0.85
Florence	1.2	70	56	24	20	26	14	13	3	1.12

Source: Galagan, D. J., and Lamson, G. G. Climate and endemic dental fluorosis. Pub. Health Rep. In press



Mean annual temperature: Arizona, 70°F.; Midwest 50°F. Number of children: Arizona, 339; Midwest, 3,249.

FIGURE 4.—*Relationship between fluoride concentration of public water supplies and dental caries experience of children, ages 12–14, in 6 Arizona and 10 midwest communities.*

Ordinarily, caries experience rates are not determined for such small numbers of children as were available in these age groups in the Southwest, and deductions from such data must be considered with reservations.

The DMF rates for the Southwestern children, presented in column 5, range from 200 DMF permanent teeth per 100 children in Casa Grande to 356 DMF teeth in Florence. The attack rates in all the Arizona communities are low. Yuma and Tempe children who have been exposed to fluoride concentrations of 0.4 and 0.5 p.p.m., respectively, demonstrate caries experience comparable to that observed in Midwestern children consuming water containing fluoride concentrations of about 1.0 p.p.m.

These data are presented graphically in figure 4. The similarity between the upper limits of the Midwestern rates and the lower limits of the Arizona rates is evident. Even though determined on a limited number of observations in Arizona, these data also suggest that the children in the Southewst area are ingesting about twice as much fluoride as children in Midwestern communities where the water supplies contained comparable fluoride concentrations.

It may be assumed that the additional fluoride consumed by the Arizona children is because of increased water consumption. This appears logical on the basis of the known impact of climatic extremes on body water metabolism and the obvious differences in climate between the two areas.

CLIMATE AND WATER CONSUMPTION

It has been estimated that about one-third of the total fluid intake of individuals is in water, the balance in other fluid substances or food (*19, 20*). Except when fluids are consumed because of emotional states of a transitory nature, fluid intake results from water deficiency. The body water lost must be replaced, and, generally speaking, water intake and water loss will be equal over a 24 hour period.

The amount of water required by the body over and above the amount needed for basic physiological processes is determined largely by the body's need to maintain normal temperature. The additional intake of water is a direct reflection of the amount of water lost in perspiration in the normal process of dissipating body heat.

Body size and weight, the amount and kind of food eaten, habit patterns, and physical activity, all influence the amount of fluid required (*21*). These factors which influence metabolic heat stress obviously will vary between individuals, and they probably are responsible for the variation in fluorosis and dental caries

TABLE 3.—*Summary of the dental caries experience of 3,249 children examined in 10 Midwestern communities and 339 children examined in 6 Arizona communities, ages 12–14*

Community	Fluoride concentration	Number of children	Total number of DMF teeth	Number of DMF teeth per 100 children
1	2	3	4	5
Marion, Ohio	0.4	263	1,461	556
Elgin, Ill.	0.5	403	1,789	444
Pueblo, Colo.	0.6	614	2,528	412
Kewanee, Ill.	0.9	123	422	343
Aurora, Ill.	1.2	633	1,718	281
East Moline, Ill	1.2	152	461	303
Maywood, Ill.	1.2	171	442	258
Joliet, Ill.	1.3	447	1,443	323
Elmhurst, Ill.	1.8	170	429	252
Galesburg, Ill.	1.9	273	643	236
Yuma, Ariz.	0.4	29	71	245
Tempe, Ariz.	0.5	45	127	282
Tucson, Ariz.	0.7	167	581	348
Chandler, Ariz.	0.8	42	103	245
Casa Grande, Ariz.	1.0	22	44	200
Florence, Ariz.	1.2	34	121	356

Source: Data on Midwestern children from Dean, H. T. Epidemiological studies in the United States. In Dental caries and fluorine. Lancaster, Pa. Science Press. 1946.

Data on Arizona children from Galagan, D. J., and Lamson, G. G. Climate and dental fluorosis. Pub. Health Rep. In press.

experience commonly observed in children exposed to the same fluoride concentration. Metabolic heat gain will not vary between locality to any great extent.

On the other hand, environmental factors will vary between geographic areas. Directly and indirectly, climate plays an important part in the total impact of the environmental forces exerted on the water metabolism of the body. There are several climatic factors which may affect water intake.

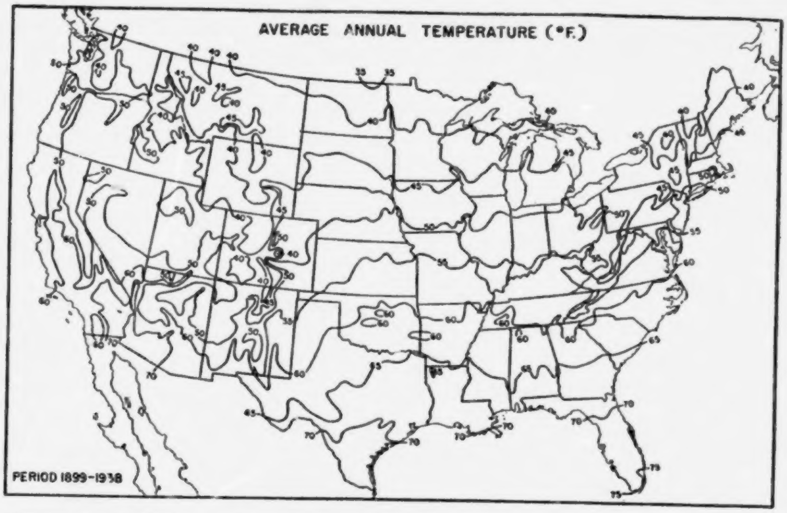


Figure 5.—*Variations in temperature throughout the continental United States. (Source: U.S. Department of Agriculture, Climates of the United States. Yearbook Separate No. 1824, Washington, D.C., 1941.)*

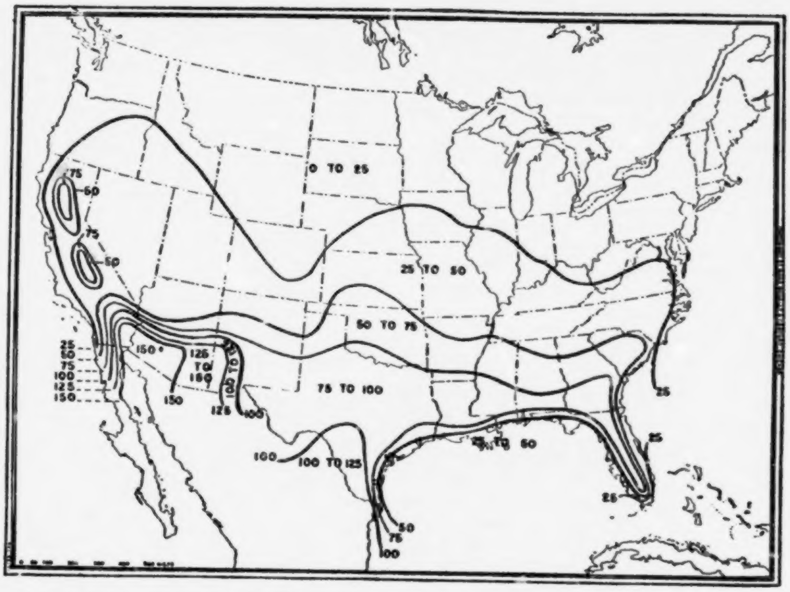


Figure 6.—*Average number of days per year temperature exceeds 90° F. (Averages based on data for 20 to 60 year periods from U.S. Department of Commerce, Weather Bureau.)*

Mean Annual Temperature

It is a physiologically demonstrated fact that increased temperatures result in an increased bodily demand for fluid (*12*). The body normally maintains an even temperature, giving off heat by radiation, conduction and evaporation, or as a result of movement of air.

Normally, skin temperature is approximately 92° F. When environmental temperatures rise above skin temperature, heat loss is accomplished by means of evaporation alone. Consequently, the water output of the body is increased in proportion to the need for increased evaporation when that temperature is exceeded. Since increased water output requires an equal increase in the amount of water ingested in order to keep the body in water balance, it follows that high temperatures will invariably result in greater water intake.

Figure 5 demonstrates the geographical pattern of mean annual temperatures in this country. It will be seen that these temperatures range from a low of 35° to 40° F. in some of the Northern states to 70° and 75° F. in the Southern states. The area immediately below the lower end of Lake Michigan and the area in Southwestern Arizona, the locations of the two groups of communities reported on, should be noted particularly.

Excessive Daytime Temperatures

Mean annual temperatures are based on the averages of the lowest and highest temperatures recorded in each 24 hour period. Since the point at which the body markedly steps up the evaporative cooling process lies between 90° and 95° F., a measure of daytime temperatures may be a more valid index of the effective temperatures to which the body is exposed. This may be particularly true in the case of children because most of their activity is during daytime hours.

At certain weather stations data are available showing the number of days the temperature exceeds 90° F. Generally speaking, there will be a direct relationship between mean annual temperature and this measurement. The distribution of the number of days the temperature exceeds 90° F. is shown in figure 6. The Arizona area with which this study is concerned experiences by far the greatest number of days during which temperatures exceed 90° F. The number of days in which the temperature passes this point in the Arizona communities is approximately 150 or more, whereas in the Midwestern communities under consideration, it is less than 25. In general those areas adjacent to large bodies of water have relatively few days with extremely high temperatures. The same is true of a large section of the Northern tier of states.

When available, measurements of such critical temperatures may be the most sensitive criterion of climatic conditions which can be used in establishing the optimum concentration of fluoride.

Radiant Heat Gain

Individuals exposed to the same air temperatures and variable amounts of direct sunlight are subject to different influences on water loss. Radiant heat gain by exposure to the sun has been specifically demonstrated to increase the amount of water lost by the body (*12*). The ground, buildings and other surrounding objects heated by the sun also contribute greatly to the body's environmental heat gain because of radiation. Therefore individuals living in areas with a large number of sunny days are exposed to an additional environmental heat stress not reflected in the usual temperature measurements. Some indication of environmental heat gain can be obtained from a measurement of the amount of available sunshine.

The variations in the percent of possible sunshine in the United States are shown in figure 7. A rather direct relationship also exists between this factor and the mean annual temperature. Again it can be seen that the most extreme conditions occur in the Southwest desert area, the more moderate exposures in the North and Northwest portions of the United States. The area in which the Arizona communities are located has approximately 80 to 85 percent of possible sunshine, whereas the Midwestern area has 50 to 60 percent. Adolph (*12*), whose studies of the influences of climate on man are exhaustive, feels that radiant heat gain is next in importance to air temperature as a means of evaluating the effect of climatic forces on water consumption.

Relative Humidity

The data on the influence of relative humidity on water intake are somewhat confusing. It is known that the amount of moisture in the air will clearly influence the exchange of water from the skin to the air. It has been demonstrated on an experimental basis that a high relative humidity will increase body water loss (*10*). By retarding evaporation, the cooling mechanism of the body appears to be made less effective. As a result the body is required to secrete more perspiration to achieve the same degree of cooling obtained by the expenditure of a smaller amount of water in a less humid atmosphere.

From a practical point of view, at high temperatures relative humidity may be considered a minor factor affecting body water loss. Adolph (*12*) was unable to determine the effect of relative humidity on water consumption in field observations in the tropics where the humidity was as high as 80 percent. If it is a factor, he feels it to be unimportant as compared to the other environmental forces which are impinging on the human body undergoing the usual climatic exposures.

Relative humidity data are not recorded in every weather station, and appear to be much less reliable than temperature data for comparative purposes. These data were available in a limited number of the study communities. The mean relative humidity of the Midwestern area is 75 percent, whereas in the Arizona communities it ranges from 30 to 35 percent. Whether these differences in relative humidity are partly responsible for the variations in the biological indices observed in the two areas is questionable.

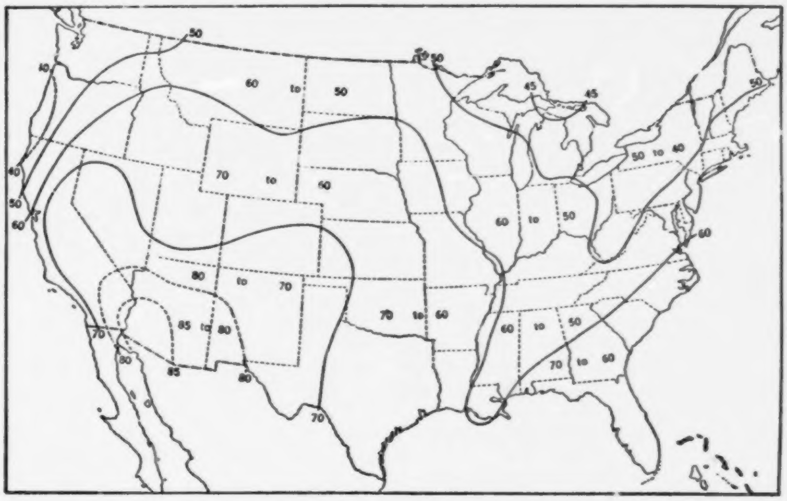


Figure 7.—*Percent of possible sunshine in the United States. (Source: Redway, J. W. Handbook of meteorology. New York, John Wiley & Sons, 1921 Reprinted by permission of the publishers.)*

Evaporation Point

Wind movement, humidity, barometric pressure and temperature all affect the speed and extent of evaporation. The speed and extent of evaporation, like relative humidity, determine the water demand of the atmosphere, but apparently they have not been demonstrated in field observations to have an effective influence on body water metabolism.

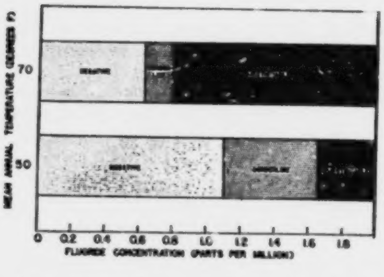
Wind Movement

Increased air motion also will aid the evaporative process. The slight variations in wind movement between geographic areas, however, have not been demonstrated to have a measurable influence on body water loss. This may be due to the fact that its effect is overshadowed by the influence of temperature. It appears that relative humidity, evaporation point and wind movement, while often felt to be important in a consideration of water intake, and certainly very important to a sense of comfort, do not influence body water metabolism to any measurable degree.

All the characteristics of weather vary with air temperature. Therefore a temperature measurement may serve as the most useful index of climate when attempting to compute the influence of environmental conditions on water intake. The general relationship between mean annual temperature and the other component weather variables further suggests that, with certain exceptions, mean annual temperature can be used as an acceptable index of climate. Its universal availability, acceptance and understanding make it a practical index. Since temperature is by far the most important weather variable influencing body water metabolism, it appears to be the index of choice for use at the present time in selecting the optimum fluoride concentration in relation to climate.

It is possible to estimate the optimum fluoride concentration range for the two areas under consideration by using mean annual temperature as the climatic index, and the community fluorosis index as the most sensitive measure of water intake.

In figure 8, a comparison of the distribution of the fluorosis indices in rela-



*Fluorosis index: Negative 0—0.4; borderline >0.4—>objectionable 0.6.

FIGURE 8.—*Relationship between mean annual temperature (50° F. and 70° F.) and fluoride concentration at which community fluorosis index indicates negative, borderline and objectionable fluorosis.** *(Source: Fig. 3.)*

tion to fluoride concentration is made for the two climatic zones. The bottom bar representing communities with mean annual temperatures of 50° F. indicates that a fluoride concentration close to 1.1 p.p.m. would be desirable, and that concentrations up to 1.7 would be acceptable. This is in accord with the present concept that the optimum concentration of fluoride is 1.0 to 1.5 p.p.m.

On the other hand, the observations made in the 70° F. communities represented by the top bar suggest that at those temperatures, 0.6 p.p.m. of fluoride is the desirable concentration, and levels up to 0.8 p.p.m. are acceptable. The rather narrow leeway available in the selection of an optimum concentration in the higher temperature zone is worthy of note.

Thus far it has generally been the practice to adjust the concentration of fluoride in fluoride-deficient water supplies to a fixed optimum concentration range without taking climate into consideration. Those areas which have altered their recommended concentrations have had to do so with little or no evidence to indicate general climatic influence on the water intake of children during their tooth-forming years.

The data presented indicate that environmental conditions do influence the water intake of infants and children. They also make it possible to determine the optimum fluoride concentration ranges in two climatic zones. It is suggested that the procedures followed may be used to explore more fully the relationship between climate and water intake in less extreme climates.

SUMMARY

1. The application of a biological index, endemic dental fluorosis, supported in part by another index, dental caries experience, in two groups of children exposed to different environmental conditions indicate that climate is an influential factor in the water consumption of infants and children.

2. The data suggest that children born and raised in areas with mean annual temperature of 70° F. consume approximately twice as much water as children living in areas with mean annual temperature of 50° F.

3. It is suggested that temperature is the most important element of climatic environment, and that mean annual temperature is the most practical measurement of climatic conditions available at the present time.

4. Fluoride concentration should be adjusted to climatic conditions. One to 1.2 p.p.m. of fluoride may be considered optimum in zones comparable to the Great Lakes area, whereas 0.6 p.p.m. may be considered equally effective in areas similar to Southern Arizona.

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AN EVALUATION OF THE GRAND RAPIDS WATER FLUORIDATION PROJECT

By John W. Knutson [1]

First, I should like to thank you for the privilege and opportunity of participating in your meeting. Second, through you, and particularly through the physicians of this city, I should like to express our deep appreciation to the people of Grand Rapids for the generous and understanding cooperation given throughout the controlled water fluoridation study which has been in operation here since January 25, 1945.

The whole world owes a debt of gratitude to a community such as Grand Rapids which was willing to be the first to take on the new in order that some of the old might be laid aside. It takes something of a venturesome courage, and faith of an extraordinary type, to do as the people of Grand Rapids did in 1944 when they approved the fluoridation study project proposed by the University of Michigan, the Michigan State Health Department, and the U.S. Public Health Service. For that faith, understanding, and cooperation, health workers and scientists will forever be obliged to you.

The Grand Rapids fluoridation project was started in 1945 with three major objectives. First, to determine the safety of the procedure; second, to determine its effectiveness as a mass method of reducing dental caries; and, third, to determine the practicability of the procedure. Among these objectives the one requiring most serious consideration under any circumstances was that of safety. It was the factor which received the closest scrutiny and assessment before the people of Grand Rapids were asked to undertake fluoridation on a study basis.

I am sure none of you would imagine that any one of the participants in the study, the University of Michigan, the Michigan State Health Department, or the U.S. Public Health Service would have proposed the project had there been any reasonable doubt about its safety from the standpoint of effects on the people of Grand Rapids. Our concern for safety then, was not that we had any doubt that a concentration of 1.0 p.p.m. of F. could be maintained in the water supply or that the people of Grand Rapids would suffer in any way. We knew that Grand Rapids had a very conscientious, highly competent director of its water works. But we didn't know how much of his conscientious attention and skill would be required to maintain the fluorine concentration at a level of 1.0 p.p.m. In other words, what were the technical difficulties in using mechanical feeders to regulate the fluorine concentration? If failures in equipment occurred, in what direction would they occur? How often and how elaborate would the fluoride tests have to be? Again, let no one think that any one of us would seriously consider exposing the population of a city of 165,000 people to a possible hazard of unknown risk.

The direct information available to us in 1944 on the dental and other physiological effects of fluoride-bearing drinking water on man was collected in areas where the water naturally contained appreciable amounts (*5*, *6*). Here I should like to say parenthetically that the only difference between natural and controlled fluoridation from a chemical standpoint is that in the first instance the fluoride accrues to the water supply through leaching of fluoride-bearing rock traversed by the water and, in the latter case, from fluoride compounds added in controlled amounts by machinery. The form of the fluorine in the water in either case is that of the fluoride ion. Therefore it seems to me of particular importance to those who are concerned about the safety of fluoridation that nature provided an abundant laboratory in which to determine its effects on man.

Here in the United States, there are 62 million people using drinking water containg 0.1 to 0.6 p.p.m. of fluorine. Another 3 million use drinking water which naturally contains 0.7 to 1.5 p.p.m. An additional million people are using drinking water with more than 1.5 p.p.m. Furthermore, I want to remind you that fluorine is a familiar substance in our dietary. Fish is relatively high in fluorine. Pablum, a widely used baby food, has or has had from 8.0 to 15.0 p.p.m. Tea leaves have a high concentration ranging from 30.0 to 60.0 p.p.m. Liver, which is prescribed as one of our health giving foods in special conditions, contains fluorine. Thus, we are not dealing with a substance which is foreign to the dietary of the American people. It is a very common one (*4*).

Basically, the proposal to control the fluoride content of the driking water represents an unwillingness to continue to accept the helter-skelter amounts added by nature. In general, communities with less than 0.6 p.p.m. do not have enough and today 800 of these communities representing over 15 million people have instituted controlled fluoridation. On the other hand, the 3 million people who have 0.7 to 1.5 p.p.m. in their drinking water have within the range of the optimum amount. Those having more than 1.5 p.p.m. should concern themselves with the problem of reducing the fluorine concentration to the optimum amount.

A relatively large number of people had for generations used naturally fluoridated drinking water, yet no scientific evidence indicated a hazard related to the use of such water where the fluorine concentration was below 1.5 p.p.m. Mottled enamel was the general sign associated with higher concentrations. This, of itself, appeared to be good presumptive evidence of the safety of 1.0 p.p.m. since several of the high-fluoride communities were in areas with university hospitals which were associated with teaching, research, and graduate medical activities such as autopsy examinations and the study of pathological specimens. A comprehensive physical examination including roentgenograms of the skeletal tissues and blood chemistry had been made of the continuous residents of Bartlett, Tex., whose drinking water contained 8.0 p.p.m. of fluorine (*3*). The results of this study indicated that mottled enamel was the only consistent symptom of excessive fluoride ingestion. There were slight indications of increased bone density in a small percentage of cases but in none of these was there any sign of interference with function. The findings on mottled enamel were in accord with the thoroughly established observation that as the fluorine concentration in the drinking water increases above 1.5 p.p.m. the percentage of cases with mottled enamel and the severity of the mottling increases.

In addition, there have been extensive animal studies on the physiological effects of fluorine. The results of these have confirmed the observation on human subjects. The finding of major importance is that the first signs of excessive ingestion of fluorine through the drinking water route are found in the teeth. The most sensitive tissues to fluorides are calcified structures and the most sensitive of these is developing enamel.

It is for this reason that a large part of the studies relating to the physiological effects of fluorides are concerned with their effect on calcified tissues. Nevertheless, the studies do include observations on the elimination of fluorides by the kidneys and the skin. They include studies on height and weight, and the incidence of bone fractures. Finally, they include detailed examinations of mortality data by cause of death and a comparison of these data for high-fluoride areas with similar data for areas having low or fluoride-free drinking water. None of these studies have presented evidence which would contraindicate controlled water fluoridation.

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[1] Presented at the Annual Meeting of the Michigan State Medical Society, Grand Rapids, Mich., Sept. 25, 1953.

The basic plans for the Grand Rapids water fluoridation project provided for the collection of base line and periodic follow-up data on dental and related conditions. Provision was also made for collecting similar data in a control city, Muskegon, Michigan, whose source of drinking water was the same as that of Grand Rapids. In addition, comparable data would be collected in Aurora, Illinois, a community whose drinking water has an optimum natural fluoride concentration of 1.2 p.p.m. Dental caries experience would be recorded as the total number of teeth with evidence of being decayed, missing, or filled (DMF for permanent teeth and def for primary teeth) (*1, 2*).

A comparison of the base line dental caries experience in permanent teeth of continuous residents of Grand Rapids (19,680 children), Muskegon (4,291 children), and Aurora (5,116 children) are shown in figure 1, by age. Note that the average number of DMF permanent teeth per child for Grand Rapids and Muskegon children, by age, were quite similar in 1945. On the other hand, Aurora children consistently had approximately two-thirds less dental caries experience.

A study of these prevalence lines (fig. 1) shows that the number of DMF teeth increases in a straight line fashion with increasing age so that Grand Rapids children aged 16 years have 13.5 DMF permanent teeth. Thus, it can be readily estimated that Grand Rapids children were accumulating 1.3 DMF permanent teeth per year in 1945 and Aurora children were accumulating 0.5 DMF teeth per year.

The next figure (fig. 2) shows the relationship of the DMF prevalence lines to each other 5 and 8 years after fluoridation was started. The downward trend in the caries experience line for Grand Rapids becomes more apparent as time after fluoridation increases. The trend is in the direction of an approach to the Aurora line which is being reached first in the younger age groups (fig. 3). This observation is in accordance with the finding that in order to obtain the full dental caries inhibiting effects of fluoridated drinking water, children must use the water from birth when the teeth begin to calcify, onward.

Inasmuch as the results of the Grand Rapids study have been disseminated during the past three years and inasmuch as they are in full accord with the results of similar, independent studies conducted in other communities, such as Newburgh, N.Y.; Brantford, Ontario; Sheboygan, Wis.; Marshall, Tex.; Evanston, Ill.; and Lewiston, Idaho, there is now virtually unanimous agreement that the fluoridation of water supplies brings about a marked reduction in dental caries experience. The reduction in terms of average number of teeth attacked by caries is of the order of 60 to 65 percent. However, this estimate is conservative since the number of surfaces affected per tooth and the degree of caries are also reduced.

It has frequently been said that the benefits of fluoridated water are limited to children below eight years of age, that is, to the period when enamel is being formed. However, this statement is not in accord with the results of an internal analysis of postfluoridation dental caries experience. For example, Grand Rapids children who were 8-years-old at the time fluoridation started are now 16-years-old. Today's 16-year-olds have 25 percent less DMF teeth per child than their counterparts had in 1945. Furthermore, as 8-year-olds in 1945 they had an average of 2.9 DMF teeth and 8 years later they have 10.1 DMF permanent teeth. Therefore, the annual incidence rate is 0.9 DMF teeth, or more than 30 percent less than the 1.3 rate observed in 1945. An extension of this type of internal analysis of the data leads to the conclusion that the benefits of water fluoridation are by no means limited to young children. Approximately one-third of the benefits can be obtained after the enamel is calcified and after the teeth have erupted. Therefore, a continuation of the exposure of the teeth to fluoridated drinking water is essential to the maintenance of maximum benefits such as those observed in the comparison of findings for adults in the Boulder-Colorado Springs study.

As is to be expected, the reduction in caries experience observed in Grand Rapids children in terms of average number of DMF permanent teeth is accompanied by a marked increase in the proportion of children whose permanent teeth are caries-free. This comparison is shown in figure 4. Note that more than three times as many children aged 6 years had one or more DMF teeth in 1945 as their counterparts have in 1953.

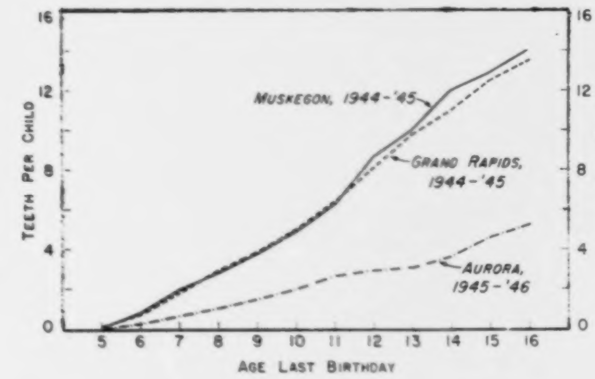
The findings on dental care expressed as filled permanent teeth indicate that the amount of dental care has remained remarkably stationary over the 8-year period of the study (fig. 5). However, maintenance of the same level of care is bringing about the highly desirable goal of closing the wide gap between dental service needs and dental care. Manifestly, the ideal situation would be reached when needs and care are equal. Fluoridated drinking water is making the approach to this ideal a practical reality in Grand Rapids.

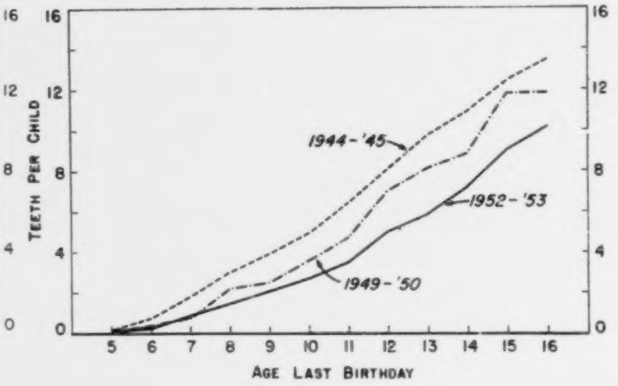
As you know, untreated dental caries is the major cause of tooth loss in children. Figure 6 shows the marked reduction in loss of permanent teeth by age, that has been accomplished in Grand Rapids during the past 8 years. This reduction is directly traceable to the reduction in dental caries experience and is not due to an increase in the level of dental care.

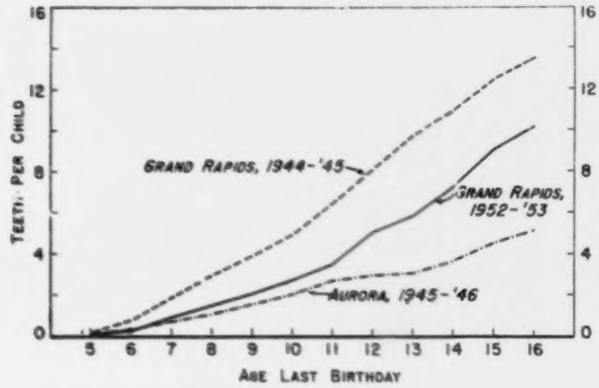
The effects of fluoridated drinking water on the prevalence of dental caries in the primary or "baby" teeth of Grand Rapids children are shown in figure 7. Note that the 1953 children aged 4, 5, and 6 years, have less than half as many def primary teeth as their counterparts had in 1945. Since exfoliation of primary teeth, both carious and sound, proceeds rapidly after age 6, a full evaluation of the benefits of fluoridated water cannot be obtained by a direct comparison of prevalence data for children who are older. Nevertheless, the prevalence lines show the differences are appreciable.

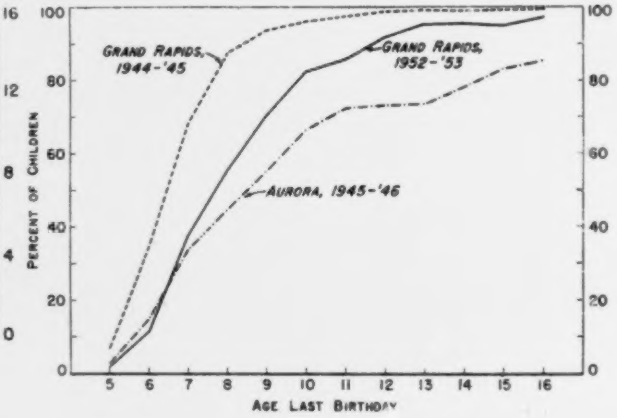
Thus, the dental health benefits which have accrued to Grand Rapids children since 1945 and which are attributed to the fluoridated drinking water are quite apparent. Fluoridation has meant appreciably less dental caries, fewer toothaches, fewer dental abscesses and accompanying infection of dental origin, and a closure of the gap between dental service needs and care. An assessment of the corollary health and economic benefits is difficult, but the absurdity of the following statement is obvious, "Nothing will be lost by postponing fluoridation and everything is to be gained by learning more about the details of the physiological action of fluorides."

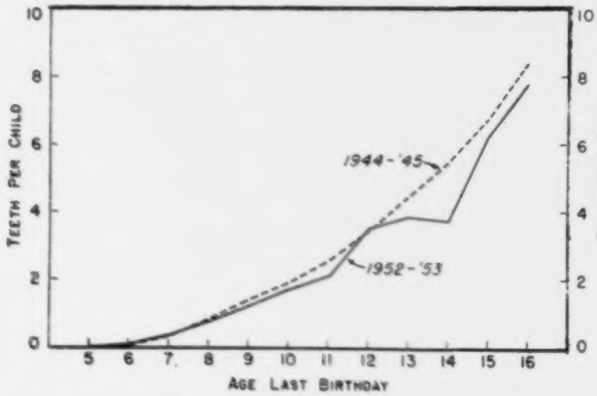
Studies on the nondental physiological effects of fluoride ingestion are continuing. To date, the results relating to the use of 1.0 p.p.m. in the drinking water have been negative. Among the continuing studies are comparisons and analyses of morbidity and mortality statistics for fluoride and non-fluoride areas. A recent analysis of mortality data compiled by the Division of Dental Public Health of the Public Health Service from 1949-1950 Census Reports are shown in figures 8, 9, and 10. These data relate to death from nephritis, cancer and heart disease, per 100,000 population, adjusted for age, race, and sex, for 28











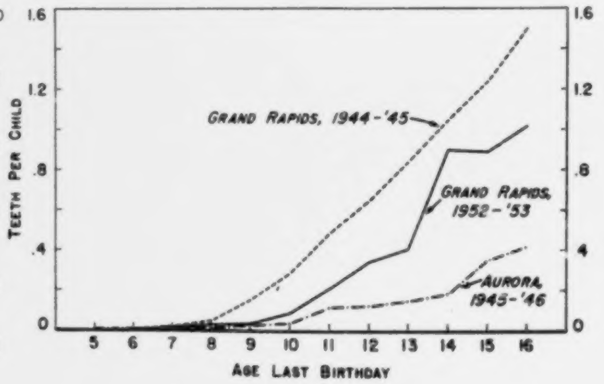


Fig. 1. DMF* permanent teeth per child for continuous residents, Grand Rapids, Muskegon and Aurora.

Fig. 2. DMF* permanent teeth per child for continuous residents in Grand Rapids.

Fig. 3. DMF* permanent teeth per child for continuous residents.

Fig. 4. Per cent of continuous resident children having one or more DMF* permanent teeth.

Fig. 5. Filled permanent teeth per child for continuous residents, Grand Rapids.

Fig. 6. Extracted permanent teeth per child for continuous residents.

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*Decayed, missing or filled.

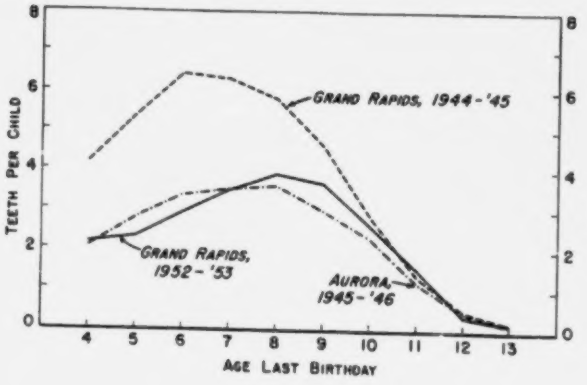


Fig. 7. DEF† Deciduous teeth per child for continuous residents.

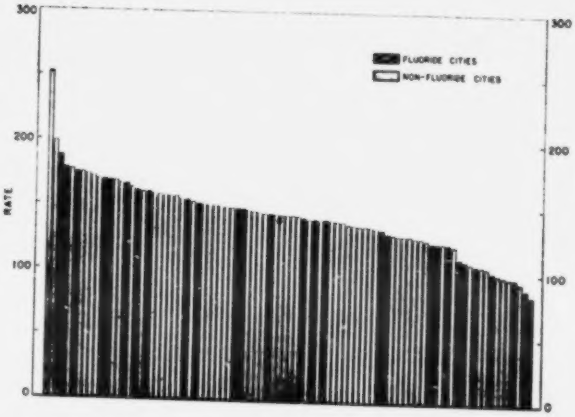


Fig. 9. Deaths* from cancer in fluoride and nonfluoride cities, 1949-1950.

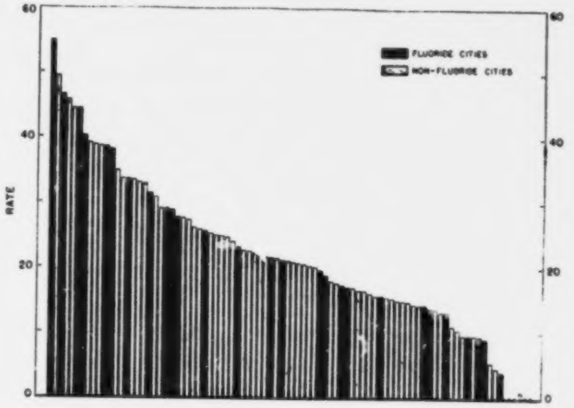


Fig. 8. Deaths* from nephritis in fluoride and nonfluoride cities, 1949-1950.

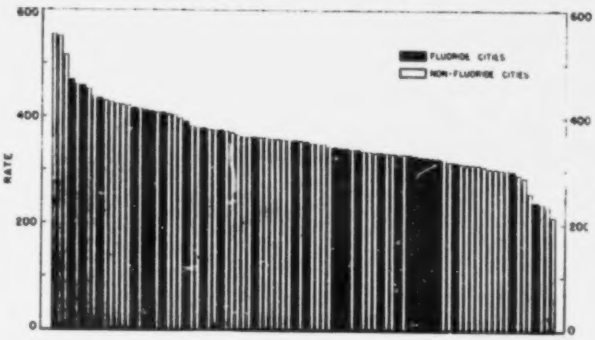


Fig. 10. Deaths* from heart disease in fluoride and non-fluoride cities, 1949-1950.

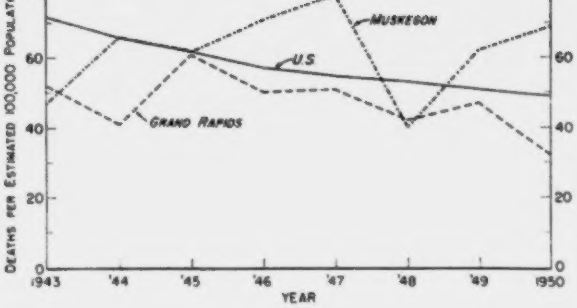
† Decayed, extraction indicated, or filled.

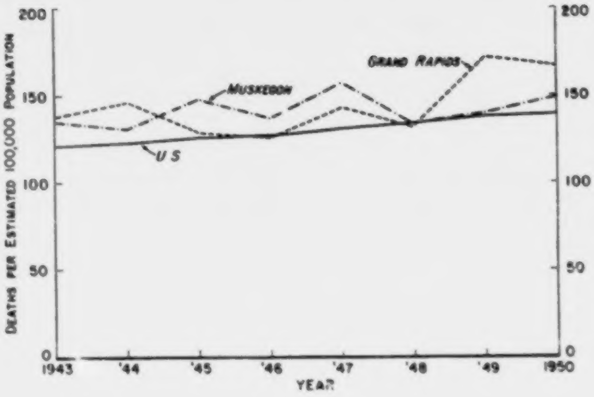
* Per 100,000 population adjusted for age, race and sex.

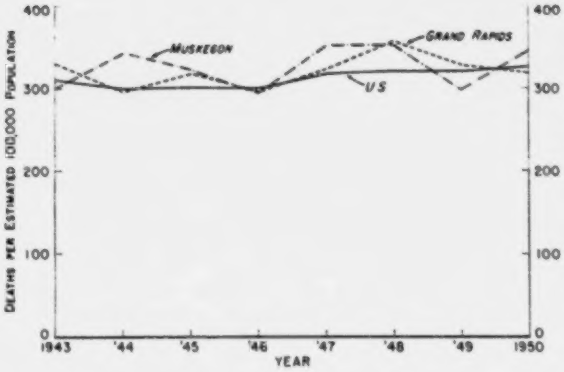
fluoride and 60 nonfluoride cities. Only cities having a population of 10,000 or greater were selected for the analysis. The fluoride cities have 0.7 or more p.p.m. of fluorine in their drinking water supplies. The nonfluoride city group is comprised of the three cities with fluoride-free water which were closest to each of the fluoride cities. Examination of the data arranged according to death rates reveals a marked homogeneity. More specifically, for nephritis (fig. 8), thirteen of the fluoride cities fall below and fifteen above the median; for cancer (fig. 9) 15 of the fluoride cities are below and 13 are above; and for heart disease (fig. 10) 16 of the fluoride cities are below and 12 are above the median.

Similar mortality data have been compiled for Grand Rapids, Muskegon, and the United States for each of 8 successive years beginning in 1943. These data are shown in figures 11, 12, and 13. In addition to mortality rates for nephritis, cancer, and heart disease, mortality rates for intracranial lesions are included (fig. 14). Examination of these data reveals the expected greater fluctuations and variability in the Grand Rapids and Muskegon annual rates than for the United States. It is of special interest that for nephritis Grand Rapids rates are consistently below those for both the U.S. and Muskegon (fig. 11). For cancer (fig. 12), for heart disease (fig. 13), and for intracranial lesions (fig. 14) both Grand Rapids and Muskegon tend to have higher rates than comparable rates for the United States. However, it is of interest to this evaluation that in each case Grand Rapids rates are either similar or lower than those for Muskegon. Muskegon did not begin fluoridating its drinking water supply until 1951 so that for each of the years covered in these comparisons, 1943 through 1950, Muskegon's drinking water was not fluoridated.

The conclusion for this evaluation is, then, that absolutely no indications or suggestions of undesirable non-dental physiological effects can be attributed to the fluoridation of the drinking water in Grand Rapids during the past eight years. The striking dental benefits have not been offset by any undesirable consequences.







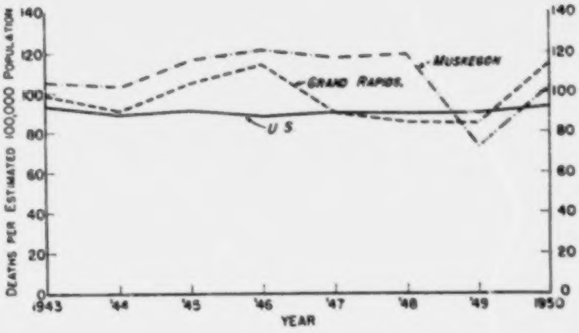


Fig. 11. Mortality rates* for nephritis in the U. S., Grand Rapids, and Muskegon, 1943-1950. (Based on population estimates).

Fig. 12. Mortality rates* for cancer in the U. S., Grand Rapids, and Muskegon, 1943-1950. (Based on population estimates).

Fig. 13. Mortality rates* for heart disease in the U. S., Grand Rapids, and Muskegon, 1943-1950. (Based on population estimates).

Fig. 14. Mortality rates* for intra-cranial lesions in the U. S., Grand Rapids, and Muskegon, 1943-1950. (Based on population estimates).

* Adjusted for age, race and sex.

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EFFECT OF FLUORIDATED PUBLIC WATER SUPPLIES ON DENTAL CARIES PREVALENCE. Tenth Year of the Grand Rapids-Muskegon Study

By FRANCIS A. ARNOLD, JR., H. TRENDLEY DEAN, PHILIP JAY, and JOHN W. KNUTSON

As early as 1942, it had been adequately demonstrated that the use of fluoride-bearing drinking waters produces a marked reduction in the incidence of dental caries. It was also known by that time that this beneficial effect occurs in populations using water supplies containing fluoride from natural sources in concentrations below the level established as the threshold for mottled enamel or endemic fluorosis (*1*).

These epidemiological studies suggested the hypothesis that the addition of fluorides to public water supplies would result in a substantial reduction in dental caries. To test this hypothesis and to evaluate the procedure as a method of caries control, several studies were begun in 1944–45. One of these studies is the Grand Rapids-Muskegon study, conducted by the Public Health Service with the cooperation of the Michigan Department of Health, the University of Michigan, and the city officials of Grand Rapids and Muskegon, Mich. This paper summarizes some of the findings from 10 years of observation in that study.

GENERAL PROCEDURE

As originally planned the Grand Rapids-Muskegon study was designed to continue for a period of 10 to 15 years. This period was selected so that observations would be comparable to those obtained in the basic epidemiological studies on children 12 through 14 years of age with a continuous history of using fluoridated water. The selection of the study areas, the methods and types of examinations, the selection of the annual study groups, and preliminary findings have been reported (*2*, *3*).

Beginning in January 1945, sodium fluoride has been added to the water supply of Grand Rapids, the principal study area. Since that time the fluoride content of the water supply has been maintained at a concentration level of 1 p.p.m. (within the range 0.9 to 1.1 p.p.m.). The water supply at Muskegon, the control area, contained less than 0.2 p.p.m. of fluoride until July 1951. At that time Muskegon began adding fluoride to its water supply to maintain a level similar to that in Grand Rapids. (In this paper, parts per million of fluoride refers to the concentration of the fluoride ion.)

To establish the caries status of the study population before fluoridation of the waters, complete oral examinations with a mouth mirror and explorer were made of virtually all children enrolled in elementary and secondary schools in Grand Rapids and Muskegon in 1944 and 1945. Each year thereafter, similar examinations have been made of selected samples of children in the two areas. (Bite-wing roentgenographic and bacteriological studies on selected samples of children will be reported in subsequent papers.) To provide an "expectancy curve," complete oral examinations were also made, in 1945 and 1946, of school children in Aurora, Ill., where it is known the water supply has contained 1.2 p.p.m. fluoride from natural sources for about 50 years.

The annual samples of the school population of Grand Rapids and Muskegon are taken from schools selected as representative of each city as a whole. The samples consist of all available children in certain grades (or in sections of the grades) in these schools. The grades are selected to yield certain age groups. The number of grades has been expanded each year so that for the 10th year of the study a representative sample of children at each age from 5 through 16 years was obtained. The grades selected for the annual examinations are as follows:

Year	*Grade*
1945	Kindergarten, 1, 8
1946	Kindergarten, 1, 4, 8
1947	Kindergarten, 1, 4, 8, 11
1948	Kindergarten, 1, 4, 8, 11
1949	Kindergarten, 1, 4, 6, 8, 11
1950	Kindergarten, 1, 2, 3, 4, 6, 8, 11
1951	Kindergarten, 1, 2, 3, 4, 6, 8, 11
1952	Kindergarten, 1, 2, 3, 4, 5, 6, 8, 11
1953	Kindergarten, 1, 2, 3, 4, 5, 6, 7, 8, 11
1954	Kindergarten through 11

Each year of the study, all available children in the selected grades (or section thereof) in each school in the study received dental examinations. Only the records of children aged 4–16 years who had used city water supplies continuously since birth are included in this report. Not included are records of children who lived outside their respective communities for more than 3 months in any one calendar year. The number of continuous resident children and their distribution by age for each year of the study are shown in table 1.

RESULTS

The amount of dental caries observed at each annual examination through 1954 in Grand Rapids and in Muskegon is shown in tables 2 and 3. There has been a striking reduction in the amount of dental caries in both the deciduous and permanent teeth. For example, in 1944 the average 6-year-old child had 6.43 def (decayed, extraction indicated, or filled) deciduous teeth; in 1954 the average child had only 2.95 def teeth. This represents a reduction of about 54 percent. Similar results were observed in the permanent teeth of children born since fluoridation started; that is those 6 to 10 years old. It should be noted also that some beneficial effect was obtained by the older children. For example, the 16-year-old children had an average of 13.50 DMF (decayed, missing, or filled) permanent teeth in 1944 and 9.95 in 1954. They were between 6 and 7 years of age when fluoridation started.

A breakdown of the dental caries experience rates for 1954 in the two cities is shown in table 4. For comparison with these results, table 5 offers the findings in 1945 among Aurora, Ill., children, who had used a naturally fluoridated water since birth. The caries experience observed among them is similar to that reported for other areas with fluorides of that concentration (*1*).

Prior to the decision to add fluoride to the water supply at Grand Rapids, it was concluded that the procedure would not produce an undesirable cosmetic effect, that is, mottled enamel. However, it was recognized that an increase in the milder, nonobjectionable forms of dental fluorosis was likely. In order to evaluate this factor fully, it is necessary to wait for observations on all permanent teeth (excluding third molars) which are calcified on fluoridated water. The ob-

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[*] The following dental officers of the Public Health Service conducted the annual dental examinations in the Grand Rapids-Muskegon study: Dr. Robert C. Likins, Dr. A. L. Russell, Dr. David B. Scott, Dr. D. E. Singleton, and Dr. Robert M. Stephan. The following dentists, formerly with the Public Health Service, also participated as examiners in the study: Dr. F. S. Loe, Los Angeles, Calif.; Dr. H. B. McCauley, Baltimore, Md.; Dr. S. J. Rusicka, Cleveland, Ohio; and Dr. Edwin M. Short, Hyattsville, Md.

TABLE 1.—*Distribution of continuous resident children examined in Grand Rapids and Muskegon, Mich., according to age, by year of examination*

Age last birthday [1]	Basic examinations, 1944–45	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954
	Grand Rapids, Mich.										
4	323	540	300	168	137	75	117	168	116	101	77
5	1, 633	1, 714	831	886	842	777	720	853	1, 087	715	529
6	1, 789	1, 186	628	663	736	697	748	750	826	1, 010	561
7	1, 806	149	82	69	55	54	438	423	422	410	75[illegible]
8	1, 647	15	216	135	138	155	501	470	444	390	567
9	1, 639	0	525	465	484	519	520	582	720	623	477
10	1, 626	0	109	108	111	125	131	141	512	499	515
11	1, 556	0	17	18	22	140	130	151	246	291	499
12	1, 685	174	85	38	60	130	200	176	211	316	260
13	1, 668	953	547	625	600	574	530	497	497	557	224
14	1, 690	273	173	196	152	153	130	128	119	111	250
15	1, 511	80	53	80	64	64	58	53	80	99	240
16	1, 107	4	3	233	245	209	177	198	191	197	198
Total	19, 680	5, 088	3, 569	3, 684	3, 646	3, 672	4, 400	4, 590	5, 471	5, 319	5, 148
	Muskegon, Mich. [2]										
4	20	-------	43	18	26	51	41	63	52	43	40
5	402	-------	321	348	422	340	359	351	487	370	381
6	462	-------	339	312	305	393	310	294	353	397	386
7	408	-------	36	42	36	30	274	223	246	209	292
8	376	-------	18	13	10	12	190	275	205	212	244
9	357	-------	213	215	199	197	227	277	348	258	275
10	359	-------	62	57	52	52	51	62	287	311	226
11	293	-------	12	10	14	146	141	139	133	175	208
12	328	-------	21	19	11	28	43	48	46	163	183
13	377	-------	197	207	208	214	173	225	178	228	243
14	369	-------	77	50	79	66	63	59	54	51	121
15	292	-------	18	44	41	34	35	21	30	35	139
16	248	-------	1	199	205	132	146	155	132	161	185
Total	4, 291	-------	1, 358	1, 534	1, 608	1, 695	2, 053	2, 192	2, 551	2, 613	2, 923

[1] See reference 1 for information regarding the effect of selection of sample by grades on specific age groups.

[2] The basic examinations in Muskegon were not done until late spring of 1945; therefore, no examinations were made in the fall of 1945.

servations to date give evidence of only a slight increase (0.24 percent in 1944; 0.36 percent in 1954) in the number of children with the milder forms of fluorosis, which are not objectionable from an esthetic or cosmetic standpoint.

DISCUSSION

From the results obtained in Grand Rapids after 10 years of water fluoridation, it is quite clear that this procedure is remarkably effective in reducing the incidence of dental caries. These observations are in accord with the results of similar studies conducted under separate auspices (*4–6*). The scientific evidence is conclusive, therefore, that water fluoridation is an effective public health procedure for producing a substantial reduction in the incidence of dental caries.

According to this study, the beneficial effects of fluoridated water are not confined to persons drinking the water since birth. The results suggest that some benefit was obtained by persons whose teeth had already formed or erupted when they started drinking fluoridated water. The effects on the teeth of adults in these cities have not as yet been ascertained. However, the fact that a reduction in caries was observed for teeth which had already been calcified when fluoridation was started indicates that some beneficial effect may be gained by older age groups.

The possibility of an increase in dental fluorosis in a community after fluoridation has received considerable discussion. After 10 years of fluoridation in Grand Rapids, the percentage of children classed as having fluorosis has increased, but, as anticipated, this increase is confined to the milder forms. As pointed out previously (7), the signs of the milder forms of fluorosis caused by ingestion of water containing 1 p.p.m. fluoride as a rule do not appear on the anterior teeth. It is the plan of this study to

TABLE 2.—*Average number of def*[1] *deciduous teeth per child*[2] *in Grand Rapids and Muskegon, Mich., by year of examination*

Age last birthday	Basic examinations, 1944–45	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954
	Grand Rapids, Mich.										
4	4.19	5.40	3.43	3.19	3.02	2.75	2.46	2.13	2.17	2.06	2.12
5	5.37	6.15	5.08	3.89	4.03	3.27	2.50	2.27	2.32	2.29	2.50
6	6.43	6.98	5.73	5.38	4.78	4.59	3.73	2.98	2.93	2.92	2.95
7	6.29	7.66	6.11	5.84	5.20	4.83	5.72	4.03	3.48	3.10	3.26
8	5.78	8.00	5.10	5.07	4.88	4.75	4.91	4.12	3.89	3.48	3.31
9	4.59	-------	4.45	4.11	4.43	4.41	4.23	3.86	3.66	3.35	3.00
10	2.84	-------	2.84	3.16	3.06	2.86	2.36	2.43	2.61	2.38	2.35
11	1.35	-------	2.12	2.78	1.77	1.19	1.16	1.35	1.51	1.90	1.32
12	.47	.28	.13	.11	.25	.35	.25	.30	.34	.44	.44
13	.18	.13	.14	.14	.17	.10	.15	.12	.17	.19	.18
	Muskegon, Mich.[3]										
4	5.05	-------	3.44	4.67	4.39	4.41	5.32	4.46	4.35	3.44	3.03
5	6.82	-------	5.86	5.05	5.55	5.56	5.65	5.25	5.39	4.42	3.98
6	7.17	-------	6.24	6.18	6.06	5.99	6.02	5.67	5.75	5.71	4.85
7	6.66	-------	6.83	5.95	6.92	6.33	5.83	5.77	5.67	5.46	5.35
8	6.06	-------	4.83	3.85	4.80	6.08	5.06	5.32	5.28	4.95	4.98
9	4.89	-------	4.32	4.34	4.71	4.48	4.09	4.17	4.36	4.29	3.81
10	3.08	-------	3.15	3.67	2.79	2.77	3.49	2.86	2.69	2.96	2.75
11	1.33	-------	1.67	2.90	.64	1.21	1.09	1.46	1.20	1.38	1.42
12	.42	-------	.14	.37	.64	.68	.61	.31	.54	.32	.61
13	.23	-------	.29	.17	.11	.11	.13	.15	.08	.21	.12

[1] Decayed, extraction indicated, or filled deciduous teeth. A decayed and filled tooth is counted only once.
[2] See table 1 for small numbers involved in some instances.
[3] The basic examinations in Muskegon were not done until late spring of 1945; therefore, no examinations were made in the fall of 1945.

continue the observations to evaluate this factor fully. Thus far, however, the ingestion of the Grand Rapids water supply has not produced any undesirable cosmetic effect in the form of objectionable dental flourosis on the anterior teeth.

SUMMARY

The results of the Grand Rapids-Muskegon study after 10 years of observation indicate that the adjustment of the fluoride content of a communal water to an optimal level (approximately 1 p.p.m. fluoride) will produce the following effects:

1. A striking reduction in the prevalence of dental caries in the deciduous teeth. At the peak of prevalence, namely 6 years of age, the caries rate for the deciduous teeth was reduced by about 54 percent.

2. A marked reduction in the prevalence of dental caries in the permanent teeth. In children born since fluoridation was put into effect, the caries rate for the permanent teeth was reduced on the average by about 60 percent.

3. Some benefit among persons whose teeth having already formed or erupted when fluoridation is begun.

4. No undesirable cosmetic effect from dental fluorosis.

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TABLE 3.—*Average number of DMF*[1] *permanent teeth per child*[2] *in Grand Rapids and Muskegon, Mich., by year of examination*

Age last birthday	Basic examinations, 1944–45	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954
	Grand Rapids, Mich.										
6	0. 78	0. 56	0. 25	0. 37	0. 26	0. 38	0 26	0. 26	0. 23	0. 12	0. 19
7	1. 89	1. 72	1. 11	1. 09	1. 04	. 76	1. 03	. 84	. 90	. 71	. 69
8	2. 95	3. 27	2. 54	2. 62	2. 30	2. 16	1. 77	1. 58	1. 50	1. 41	1. 27
9	3. 90	-------	2. 98	3. 12	2. 67	2. 48	2. 38	2. 04	2. 02	1. 83	1. 97
10	4. 92	-------	3. 70	3. 56	3. 51	3. 56	3. 17	2. 93	2. 71	2. 41	2. 34
11	6. 41	-------	4. 24	3. 56	4. 32	4. 69	4. 36	3. 67	3. 49	3. 12	2. 98
12	8. 07	9. 53	7. 62	7. 03	8. 32	7. 02	7. 10	5. 89	5. 04	4. 76	3. 87
13	9. 73	10. 76	8. 92	8. 47	8. 34	8. 11	7. 21	6. 60	5. 87	5. 12	5. 05
14	10. 95	11. 90	9. 41	9. 50	9. 41	8. 90	8. 55	8. 21	7. 23	5. 92	6. 78
15	12. 48	12. 68	11. 26	11. 94	10. 61	11. 80	10. 12	8. 91	9. 04	9. 75	8. 07
16	13. 50	13. 00	9. 33	12. 47	13. 50	11. 83	11. 35	11. 06	10. 14	9. 53	9. 95
	Muskegon, Mich.[3]										
6	0. 81	-------	0. 48	0. 66	0. 79	0. 63	0. 75	0. 80	0. 52	0. 35	0. 45
7	1. 99	-------	1. 33	1. 05	2. 19	1. 43	2. 01	1. 88	1. 66	1. 24	1. 14
8	2. 81	-------	2. 83	2. 15	3. 50	2. 58	2. 96	2. 63	2. 49	2. 66	2. 18
9	3. 81	-------	3. 29	3. 54	3. 58	3. 88	3. 89	3. 52	3. 05	3. 22	3. 16
10	4. 91	-------	4. 27	3. 60	4. 87	4. 44	4. 53	4. 32	3. 90	3. 64	3. 72
11	6. 32	-------	4. 25	4. 70	4. 71	5. 93	5. 67	5. 34	5. 04	4. 70	4. 58
12	8. 66	-------	8. 43	6. 79	7. 82	7. 21	6. 88	7. 71	7. 00	6. 53	6. 12
13	9. 98	-------	9. 02	9. 23	10. 52	9. 52	9. 58	9. 36	8. 71	8. 20	7. 98
14	12. 00	-------	11. 09	12. 00	12. 27	11. 08	12. 11	11. 36	10. 06	10. 35	10. 74
15	12. 86	-------	11. 17	12. 89	12. 66	10. 32	10. 94	12. 38	11. 57	11. 69	11. 19
16	14. 07	-------	19. 00	12. 77	14. 31	12. 51	13. 91	13. 16	12. 36	11. 48	12. 55

[1] Decayed, missing, or filled permanent teeth. A decayed and filled tooth is counted only once.

[2] See table 1 for small numbers involved in some instances.

[3] The basic examinations in Muskegon were not done until the late spring of 1945; therefore, no examinations were done in the fall of 1945.

TABLE 4.—*Dental caries in deciduous and permanent teeth of continuous resident children of Grand Rapids and Muskegon, Mich., as observed in the 1954 examination*

Age last birthday	Number of teeth per child						Percent of caries-free children[4]
	Deciduous teeth		Permanent teeth				
	Filled	Total def[1]	Decayed	Missing[2]	Filled	Total DMF[3]	
	Grand Rapids, Mich.						
4	0.68	2.12	----	----	----	----	----
5	1.14	2.50	0.01	----	0.01	0.02	99.4
6	1.30	2.95	.11	----	.09	.19	89.3
7	1.45	3.26	.36	----	.35	.69	66.8
8	1.42	3.31	.52	0.02	.77	1.27	49.4
9	1.30	3.00	.74	.04	1.26	1.97	33.1
10	.98	2.35	.73	.07	1.63	2.34	26.6
11	.63	1.32	.78	.10	2.19	2.98	16.8
12	.12	.44	1.14	.26	2.55	3.87	13.5
13	.04	.18	1.56	.44	3.23	5.05	10.7
14	----	----	2.13	.52	4.36	6.78	5.6
15	----	----	2.08	1.02	5.23	8.07	1.2
16	----	----	1.96	1.35	6.90	9.95	2.0
	Muskegon, Mich.[5]						
4	1.18	3.03	----	----	----	----	----
5	.98	3.98	0.03	----	----	0.03	98.4
6	1.64	4.85	.33	0.00	0.13	.45	79.8
7	1.96	5.35	.74	0	.41	1.14	49.7
8	2.03	4.98	1.23	.06	.95	2.18	27.5
9	1.60	3.81	1.29	.14	1.80	3.16	14.5
10	1.14	2.75	1.44	.23	2.20	3.72	5.7
11	.44	1.42	1.79	.32	2.67	4.58	4.3
12	.14	.61	2.05	.42	3.85	6.12	4.4
13	.06	.12	2.47	.72	4.97	7.98	1.6
14	----	----	4.31	1.39	5.31	10.74	0
15	----	----	3.55	1.42	6.51	11.19	1.4
16	----	----	2.78	1.42	8.77	12.55	1.1

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[1] Decayed, extractions indicated, or filled deciduous teeth.

[2] Includes teeth listed as "remaining roots" and teeth destroyed beyond any possible repair.

[3] Decayed, missing, or filled permanent teeth; each tooth is counted only once for this total. A tooth that has both a filled and a carious surface is included in both the "Decayed" and the "Filled" columns.

[4] Permanent teeth only.

[5] These children had already received the beneficial effects of 3 years of water fluoridation (see text).

Table 5.—*Dental caries in deciduous and permanent teeth of continuous resident children of Aurora, Ill., as observed in the 1945–46 examination period*

Age last birthday	Number children examined	Deciduous teeth		Permanent teeth				Percent of caries-free children [4]
		Filled	Total def [1]	Decayed	Missing [2]	Filled	Total DMF [3]	
		Number of teeth per child						
4	30	0. 10	2. 07	--------	--------	--------	--------	--------
5	407	. 25	2. 79	0. 06	--------	--------	0. 06	97. 3
6	473	. 38	3. 36	. 27	0. 00	0. 02	. 28	84. 8
7	516	. 44	3. 51	. 68	. 01	. 04	. 71	66. 1
8	469	. 54	3. 60	. 95	. 01	. 11	1. 04	55. 2
9	368	. 49	2. 98	1. 27	. 06	. 27	1. 52	44. 8
10	397	. 27	2. 28	1. 55	. 09	. 51	2. 02	33. 5
11	383	. 15	1. 18	2. 03	. 23	. 69	2. 67	27. 7
12	401	. 05	. 43	2. 06	. 22	. 96	2. 95	26. 9
13	401	. 01	. 13	2. 10	. 24	1. 20	3. 09	26. 7
14	433	--------	--------	2. 05	. 31	1. 58	3. 64	21. 7
15	467	--------	--------	2. 37	. 50	2. 05	4. 54	16. 9
16	371	--------	--------	2. 32	. 53	2. 71	5. 19	14. 6

[1] Decayed, extraction indicated, or filled deciduous teeth.

[2] Includes teeth listed as "remaining roots" and teeth destroyed beyond any possible repair.

[3] Decayed, missing, or filled permanent teeth; each tooth is counted only once for this total. A tooth that has both a filled and a carious surface is included under both the "Decayed" and the "Filled" columns. (Note that this table differs from table 2, reference 2.)

[4] Permanent teeth only.

CLINICAL AND ROENTGENOGRAPHIC EXAMINATIONS FOR DENTAL CARIES IN GRAND RAPIDS, MICH.

By Richard L. Hayes, H. Berton McCauley, and Francis A. Arnold, Jr.

Dental caries experience may be estimated by direct clinical observation, by roentgenography, or by a combination of the two methods. Direct observation is most convenient and is therefore most frequently employed, but the demonstration that bite-wing roentgenography is valuable for early detection of caries in the approximal surfaces of the teeth (*1–7*) has led to its use in several studies (*8–14*), including some in which it was the only method used. The combined technique, direct observation plus bite-wing roentgenography, consistently yields a higher estimate of caries prevalence than direct observation alone (*7, 10–12*); therefore, some observers have suggested that X-rays are essential for dental surveys designed to determine the efficacy of caries control measures (*8, 12, 15*).

In Grand Rapids, Mich., a study to evaluate fluoridation of a municipal water supply as a caries control measure has been in progress since 1944. As part of this study, an investigation was undertaken to determine whether or not supplementing direct observation with roentgenographic examination would affect the conclusions based on direct observation alone. Caries experience was estimated separately by direct observation and by bite-wing roentgenography. The data were then combined in such manner that, if the conclusions were affected, it would be evident, and if not, the finding would be unequivocal. Hence, the method selected for combining the data gives the greater weight to the roentgenographic findings and presents the direct observation findings adversely. The roentgenograms were used also to estimate the depth of the carious lesions.

METHODS

Each year since the start of fluoridation in Grand Rapids in January 1945, selected samples of school children have received direct observation examinations for dental caries. The examinations are performed by several dentists, using a mouth mirror, an explorer, and artificial illumination (*16, 17*).

In 1946, 1947, and 1953 (approximately 21, 33, and 105 months after fluoridation was begun), the annual dental examinations of children in the first, fourth, and eighth grades of four selected schools were supplemented by bite-wing X-rays.

TABLE 1.—*Mean number of carious teeth per child as estimated by direct observation and by direct observation plus X-ray examination, Grand Rapids, Mich., 1946–47 and 1953*

Type of examination	1946–47		1953		Difference	Standard error
	Mean	Standard error	Mean	Standard error		
	def[1] deciduous molars (ages 5–7)					
Direct observation only	4.73	0.27	2.82	0.21	1.91	0.31
Direct observation plus:						
Minimum X-ray findings	5.67	.23	4.06	.23	1.61	.35
Maximum X-ray findings	6.19	.21	4.92	.23	1.27	.33
	DMF[2] first permanent molars (ages 8–10)					
Direct observation only	2.77	0.16	1.86	0.13	0.91	0.22
Direct observation plus:						
Minimum X-ray findings	2.78	.16	1.93	.12	.85	.21
Maximum X-ray findings	3.04	.14	2.34	.12	.70	.19
	DMF[2] permanent teeth (ages 12–14)					
Direct observation only	9.66	0.45	5.44	0.36	4.22	0.64
Direct observation plus:						
Minimum X-ray findings	10.96	.46	6.50	.41	4.46	.69
Maximum X-ray findings	13.26	.50	8.73	.52	4.53	.78
	DMF[2] premolars (ages 12–14)					
Direct observation only	1.51	0.18	0.38	0.09	1.13	0.22
Direct observation plus:						
Minimum X-ray findings	1.90	.20	.73	.11	1.17	.24
Maximum X-ray findings	3.34	.22	2.08	.20	1.26	.31
	DMF[2] first permanent molars (ages 12–14)					
Direct observation only	3.93	0.04	3.17	0.12	0.76	0.14
Direct observation plus:						
Minimum X-ray findings	3.96	.03	3.30	.11	.66	.13
Maximum X-ray findings	3.98	.02	3.43	.10	.55	.11

[1] Decayed, extraction indicated, or filled.
[2] Decayed, missing, or filled.

The X-ray examinations followed the regular examinations by a period varying from a few days to a month. Left and right posterior bite-wing radiographs were made for every pupil. For each fourth-grade child (8–10 years of age), one anterior bite-wing X-ray was made to show the central incisor teeth; for each eighth-grade child (12–14 years of age), three anterior bite-wing X-rays were made to show the incisor and cuspid teeth. First-grade pupils (5–7 years of age) did not receive anterior bite-wing X-rays.

Roentgenographic interpretation was limited to the approximal surfaces of the deciduous molar teeth of 5- to 7-year-old children, the central incisor and first permanent molar teeth of 8- to 10-year-old children, and the permanent teeth (excluding third molars) of 12- to 14-year-old children. Each set of roentgenograms received two independent readings. Each reading was made without reference to the clinical examination record and was recorded separately from the other.

The first step in recording the caries status of an approximal surface was to score it as carious or noncarious. An approximal surface was scored as carious if the reader could detect a discontinuity of the enamel surface or any other sign

TABLE 2.—*Mean number of carious approximal surfaces per child as estimated by direct observation and by direct observation plus X-ray examination, Grand Rapids, Mich., 1946–47 and 1953*

Type of examination	1946–47		1953		Difference	Standard error
	Mean	Standard error	Mean	Standard error		
	df [1] approximal surfaces of deciduous molars (ages 5–7)					
Direct observation only	4.48	0.12	1.92	0.20	2.56	0.43
Direct observation plus:						
Minimum X-ray findings	6.11	.12	3.79	.26	2.32	.46
Maximum X-ray findings	7.21	.11	5.14	.29	2.07	.47
	DF [2] approximal surfaces of first permanent molars (ages 8–10)					
Direct observation only	0.56	0.11	0.08	0.02	0.48	0.09
Direct observation plus:						
Minimum X-ray findings	.81	.14	.25	.05	.56	.13
Maximum X-ray findings	1.55	.16	1.02	.09	.53	.18
	DF [2] approximal surfaces of permanent teeth (ages 12–14)					
Direct observation only	5.58	0.54	1.90	0.32	3.68	0.68
Direct observation plus:						
Minimum X-ray findings	8.60	.68	3.86	.45	4.74	.87
Maximum X-ray findings	13.07	.77	7.75	.66	5.32	1.08
	DF [2] approximal surfaces of premolars (ages 12–14)					
Direct observation only	0.63	0.13	0.09	0.03	0.54	0.14
Direct observation plus:						
Minimum X-ray findings	1.38	.22	.53	.11	85	.25
Maximum X-ray findings	3.43	.31	2.33	.26	1.10	.41
	DF [2] approximal surfaces of first permanent molars (ages 12–14)					
Direct observation only	1.85	0.16	0.75	0.11	1.10	0.21
Direct observation plus:						
Minimum X-ray findings	2.45	.18	1.37	.14	1.08	.24
Maximum X-ray findings	3.34	.19	2.46	.19	.88	.27

[1] Decayed or filled deciduous teeth.
[2] Decayed or filled permanent teeth.

of caries, such as an abnormal radiolucency at the dentino-enamel junction. These surfaces were designated as the maximum number of carious surfaces detectable by X-ray. Each carious surface was then further examined, and a separate notation was made for each one with signs of caries of the dentin. Surfaces with dentinal caries were designated as the minimum number of carious surfaces demonstrable by X-ray. Radiolucencies contiguous to restorations were not recorded, but restorations were considered caries of both the enamel and the dentin.

After the completion of all X-ray interpretations, a master record of the roentgenographic findings was made for each child. This record, which contained the notations from each of the independent readings, was used in combining the radiographic and clinical findings. To give greater weight to the radiographic findings, an approximal surface was given a roentgenographic diagnosis of caries if it was scored as carious at either reading.

Two more records were then made for each child: one for direct observation findings plus minimum X-ray findings; the other for direct observation findings plus maximum X-ray findings. Surfaces negative for caries according to direct observation but positive according to X-ray examination were added to the direct observation findings. Surfaces

Table 3.—*Number of continuous resident children examined with bite-wing X-rays and mean age of children in each age group, Grand Rapids, Mich., 1946–47 and 1953*

Age group (years)	1946–47				1953			
	Boys	Girls	Total	Mean age	Boys	Girls	Total	Mean age
5–7	58	50	108	6. 2	70	71	141	6. 4
8–10	50	41	91	9. 1	89	79	168	9. 4
12–14	59	54	113	13. 3	55	60	115	13. 3

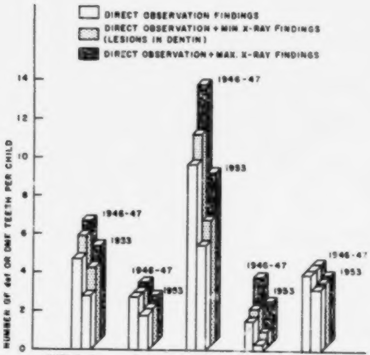

Figure 1.—*Teeth with caries experience, continuous resident children in Grand Rapids, Mich., 1946–47 and 1953.*

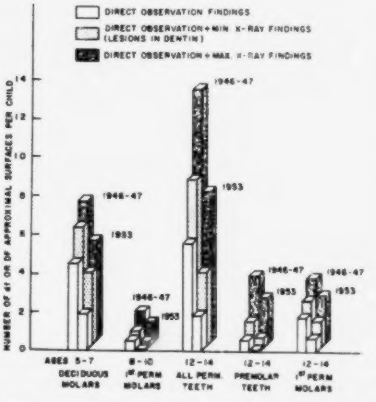

Figure 2.—*Approximal surfaces with caries experience, continuous resident children in Grand Rapids, Mich., 1946–47 and 1953.*

positive according to direct observation were considered carious at both levels of penetration, regardless of the X-ray findings.

Only children designated "continuous residents," that is, those who had consumed water from the Grand Rapids supply continually since birth and who had not been absent from that community longer than 3 months in any calendar year, were included in the study, although others were examined.

Table 4.—*Mean number of teeth per child subject to the risk of caries, Grand Rapids, Mich., 1946–47 and 1953*

Type of teeth and age group	1946–47	1953
Deciduous molars: 5–7	7. 63	7. 77
Permanent teeth:		
8–10	14. 03	13. 76
12–14	26. 80	26. 56
Premolars: 12–14	7. 60	7. 48

Of those who received X-ray examination in addition to the regular examination, 736 (312 in 1946 and 1947, and 424 in 1953) met the residence requirements. As there was no essential difference in the caries experience of the 167 radiographed in 1946 and the 145 in 1947, the data for these 2 years were combined.

The representativeness of the X-ray sample was determined by comparing the caries experience of the children in this sample, as determined by direct observation of the teeth, with that of their age peers in the entire sample of children examined in 1946–47 and 1953 (*18*). Between 1946–47 and 1953 both samples displayed substantial decreases in the number of def (decayed, indicated for extraction, or filled) deciduous teeth and in the number of DMF (decayed, missing, or filled) permanent teeth. Both samples yielded essentially the same results.

Support for Clinical Findings

Supplementation of the dental examinations with bite-wing X-rays did not change the basic observation that decreases in dental caries occurred among the Grand Rapids children between 1946–47 and 1953. The mean numbers of carious teeth were smaller in 1953 than in 1947 whether estimated by direct observation alone or by direct observation plus X-ray examination (table 1 and fig. 1). The same was true for carious approximal surfaces (table 2 and fig. 2). Inasmuch as the changes in caries experience for children in this study as determined by direct observation alone were essentially the same as those for all the children in their age groups, it is likely that the rest of the first-, fourth-, and eighth-grade children would provide similar X-ray findings.

The reduction in caries between 1946–47 and 1953 was statistically significant, as can be seen by an inspection of the standard error terms (last column in tables 1 and 2). The reduction cannot be explained by differences in the age or sex distribution (table 3) or by variations in the number of teeth at risk (table 4) since these were all essentially the same for the two periods. The between-method variation in the magnitude of the differences between the 1946–47 and 1953 caries rates might be attributed to chance.

The findings suggest, both numerically and proportionally, that there was little variation in the effectiveness of the clinical examinations between 1946–47 and 1953. The maximum X-ray increments for 12- to 14-year-old children were 3.60 DMF teeth per child in 1946–47 and 3.29 in 1953. If the mean number of teeth in eruption (table 4) is used as the denominator, X-rays revealed the first evidence of caries for 13 percent of the teeth in eruption in 1946–47 and for 12 percent in 1953. Selection of the denominator for these and other proportions is based on Hill's advice concerning numerators, denominators, and proper use of the risk factor (*19*).

New Information From X-Rays

The X-ray data revealed that most of the decrease in caries of the approximal surfaces occurred among the deeper lesions (those involving dentin) and that there was little or no change in the number of shallow lesions (those limited to the enamel). These findings are shown in table 5, where depth of lesion as determined by X-ray is related to the number of approximal surfaces present. The denominators are the numbers of surfaces at risk; they are thus corrected for the slight variation in the number of teeth in eruption but not for any possible variation in time of eruption. Extracted teeth were excluded since there was no way of determining the caries experience of their approximal surfaces.

The difference between the 1946–47 and 1953 caries patterns with regard to depth of lesion is consistent with the hypothesis that fluoride retards the development of dental caries. A reasonable explanation for the data is that some enamel lesions did not progress to dentinal lesions and that many enamel lesions did not occur or at least did not develop to a size detectable by practical methods. (Some enamel lesions might have been replaced by microscopic lesions of the type described by Burket (*20*); this study did not investigate these lesions). The nonappearance of the shallow lesions is also consistent with the hypothesis that fluoride actually prevents the inception of caries. The alternative hypothesis that fluoride selectivity prevents caries of the dentin is not supported by these data, as this hypothesis would require decreases in dentinal caries to be balanced by increases in enamel caries.

Effect on DMF Studies

Bite-wing roentgenography produced less change in estimates of caries experience of teeth (DMF or def) than that of approximal surfaces (DF or df). This can be seen by comparing parallel radiographic increments of these scores in figures 1 and 2. The lower sensitivity of DMF estimates is related to the high susceptibility of children's teeth to occlusal caries (*21*), the relative ease of detecting occlusal lesions by direct observation (*2*), and the fact that a tooth is counted only once in arriving at a DMF score. Only radiographic evidence of caries in a tooth deemed to be free of caries on direct observation would add to a DMF score.

The methods selected for combining the direct observation and roentgenographic data did, in fact, present the direct observation findings adversely, as can be seen in figures 1 and 2. As shown in tables 6 and 7, different methods of using the two independent X-ray readings would have led to the same conclusions: that there was a substantial decrease in dental caries experience and that this decrease occurred primarily among the deeper lesions. The essential conclusion—that there was a substantial decrease in dental caries experience—was reached without the X-rays.

SUMMARY

Bite-wing roentgenography was used to supplement the regular dental examinations of 736 continuous resident children of Grand Rapids, Mich., in 1946–47 and 1953. Findings from direct observation indicated that the children in the X-ray sample were representative of their age peers in the entire sample of children examined in those years.

The roentgenograms were read for radiolucencies of dentin and for discontinuities of the enamel surface. For the

TABLE 5.—*Caries of approximal surfaces, by depth of lesion as determined by X-rays, Grand Rapids, Mich., 1946–47 and 1953*

Year(s)	Number of approximal surfaces present	Number of carious surfaces			Number of caries-free surfaces	Number of carious surfaces per 100 surfaces present		
		Total	Caries of dentin	Caries of enamel only		Caries of dentin	Caries of enamel only	Caries free
	Deciduous molars (ages 5–7)							
1946–47	1, 648	756	622	134	892	37. 7	8. 1	54. 1
1953	2, 190	705	503	202	1, 485	23. 0	9. 2	67. 8
	First permanent molars (ages 8–10)							
1946–47	712	131	63	68	581	8. 8	9. 6	81. 6
1953	1, 344	171	38	133	1, 173	2. 8	9. 9	87. 3
	Premolars (ages 12–14)							
1946–47	1, 700	382	144	238	1, 318	8. 5	14. 0	77. 5
1953	1, 692	266	59	207	1, 426	3. 5	12. 2	84. 3
	First permanent molars (ages 12–14)							
1946–47	696	355	240	115	341	34. 5	16. 5	49. 0
1953	838	270	146	124	568	17. 4	14. 8	67. 8

primary purpose of testing the effect of X-ray findings on conclusions reached by direct observation alone, the X-rays were read twice and any surface scored as carious at either reading was added to the directly observed estimate of caries.

There were wide but consistent variations in total caries experience according to the three methods of estimation, but all estimates demonstrated a significant decrease in total caries experience between 1946–47 and 1953. The decrease in caries appeared to be greater for deeper lesions than for shallow lesions. This was demonstrated by analysis of roentgenographic data without reference to the regular dental examinations and was independent of the method used to combine the two X-ray readings. The decrease in the deeper lesions without a balancing increase in the shallow lesions suggests that fluoride retards the development of caries and that it also prevents the inception of caries.

From this study, we draw the following conclusions:

1. Roentgenographic findings support the clinical findings that fluoridation of a municipal water supply is an effective caries control measure.

2. Estimates of dental caries experience expressed as the number of decayed, missing, or filled teeth are less sensitive to modification by roentgenographic findings than are estimates of caries expressed as the number of decayed or filled approximal surfaces.

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TABLE 6.—*Approximal surfaces with dentinal caries, by individual and by combined roentgenographic readings, Grand Rapids, Mich., 1946–47 and 1953*

Criteria for acceptance of lesions	1946–47		1953		Change in caries experience:[1] Surfaces per 100 surfaces present
	Number of carious surfaces	Number of carious surfaces per 100 surfaces present	Number of carious surfaces	Number of carious surfaces per 100 surfaces present	
	Deciduous molars (ages 5–7)				
Either reading	622	37.7	503	23.0	−14.7
Reading 1 only	595	36.1	479	21.8	−14.3
Reading 2 only	600	36.4	479	21.9	−14.5
Both readings	573	34.8	452	20.6	−14.2
	First permanent molars (ages 8–10)				
Either reading	63	8.8	38	2.8	−6.0
Reading 1 only	56	7.9	34	2.5	−5.4
Reading 2 only	61	8.6	32	2.4	−6.2
Both readings	54	7.6	28	2.1	−5.5
	Premolars (ages 12–14)				
Either reading	144	8.5	59	3.5	−5.0
Reading 1 only	125	7.4	49	2.9	−4.5
Reading 2 only	135	7.9	53	3.1	−3.8
Both readings	116	6.8	43	2.5	−4.3
	First permanent molars (ages 12–14)				
Either reading	240	34.5	146	17.4	−17.1
Reading 1 only	227	32.6	136	16.2	−16.4
Reading 2 only	226	32.5	137	16.4	−16.1
Both readings	213	30.6	127	15.2	−15.4

[1] Minus indicates a decrease in 1953.

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TABLE 7.—*Approximal surfaces with caries limited to the enamel, by individual and by combined roentgenographic readings, Grand Rapids, Mich., 1946–47 and 1953*

Criteria for acceptance of lesions	1946–47		1953		Change in caries experience:[1] Surfaces per 100 surfaces present
	Number of carious surfaces	Number of carious surfaces per 100 surfaces present	Number of carious surfaces	Number of carious surfaces per 100 surfaces present	
	Deciduous molars (ages 5–7)				
Either reading	134	8. 1	202	9. 2	+1. 1
Reading 1 only	116	7. 0	181	8. 3	+1. 3
Reading 2 only	125	7. 6	200	9. 1	+1. 5
Both readings	106	6. 4	178	8. 1	+1. 7
	First permanent molars (ages 8–10)				
Either reading	68	9. 6	133	9. 9	+0. 3
Reading 1 only	66	9. 3	125	9. 3	0
Reading 2 only	63	8. 8	118	8. 8	0
Both readings	61	8. 6	110	8. 2	−. 4
	Premolars (ages 12–14)				
Either reading	238	14. 0	207	12. 2	−1. 8
Reading 1 only	231	13. 6	184	10. 9	−2. 7
Reading 2 only	204	12. 0	196	11. 6	−. 4
Both readings	198	11. 6	173	10. 2	−1. 4
	First permanent molars (ages 12–14)				
Either reading	115	16. 5	124	14. 8	−1. 7
Reading 1 only	115	16. 5	114	13. 6	−2. 9
Reading 2 only	101	14. 5	118	14. 1	−. 4
Both readings	101	14. 5	108	12. 9	−1. 6

[1] Minus indicates a decrease in 1953; plus, an increase.

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ORAL HEALTH STUDY IN CHILDREN OF SUBURBAN WASHINGTON, D. C.

By A. L. Russell

This is a preliminary report on a study of oral health in a population of children as the population becomes progressively less susceptible to dental caries following fluoridation of its community water. The study is not designed as a test of the fluoride-dental caries relationship; the study plan assumes that this relationship is fully established and that a progressive inhibition of dental caries will, in fact, occur. The study's broad and long-term objectives are to augment the descriptive epidemiology of dental caries and to lay a foundation for a descriptive epidemiology of periodontal disease as it first appears in relatively young persons. In addition the study group has been and will be utilized, as a population of known status and background, for short-term observation of pertinent phenomena. Two such reports, based in part upon this population, have already been published (*1,2*).

The present report is limited to observations on dental caries. It describes the study population, criteria and methods of examination, and the status of the group at the time of first examination in 1952 and includes summary data from examinations in 1953 and 1954. Evidence is presented to support other findings that an inhibition of dental caries in children will follow use of a fluoridated domestic water. The continuing study is being conducted by the National Institute of Dental Research, Public Health Service, in Prince Georges and Montgomery Counties, Md., two counties adjacent to the District of Columbia.

THE STUDY SITUATION

The population of Montgomery County in 1950 numbered 164,401 persons of whom 93.6 percent were white. The population of Prince Georges County was 194,182 persons, 88.2 percent of whom were white.

Both counties are predominantly urban. Only 6.4 percent and 5.9 percent of the residents of the respective counties lived on farms in 1950. The median number of school years completed by persons 25 years of age or older was 12.6 in Montgomery County and 12.0 in Prince Georges County. Median family income was $4,532 in the one county and $3,634 in the other (*3a*). The median value of one-family homes was $16,136 in Montgomery County and $11,696 in Prince Georges County (*3b*). Principal sources of income were Federal employment, wholesale and retail trade, construction, and service occupations. Only about 10 percent of the population were employed in manufacturing (*4*).

Compared with the United States as a whole, residents of the two counties had completed more schooling, had earned higher incomes, and had lived in more expensive homes. For the entire Nation, the median number of school years completed in 1950, by persons 25 years of age or older, was 9.3 years (*5a*). Median incomes were $2,970 for urban families, and $2,186 for rural nonfarm families, respectively (*5b*). And the median value of a one-family home, in urban and rural nonfarm areas, was $7,354 (*5c*).

About 5,000 elementary and junior high school pupils have been examined annually since the oral health study began. The Montgomery County group is taken from an area centering around the Takoma Park Junior High School, adjacent to the District of Columbia across its northeast border. The Prince Georges County group lives in the general vicinity of the Maryland Park and Suitland Junior High Schools, near the southeast border of the District. These areas were chosen as representative of suburban Washington from the socioeconomic standpoint and because their residents have been relatively nonmigratory.

Detailed data concerning the number of topical sodium fluoride treatments were obtained for each child through a schedule completed and returned by his parents. The proportion of treated children was so high in one Montgomery County neighborhood that it seemed prudent to exclude this entire group in computing caries and eruption expectancy tables.

Data for eight schools were analyzed separately by age, sex, and school. Though the proportion of filled teeth to total caries experience was uniformly higher in Montgomery than in Prince Georges County children, all children in the study seemed to have been drawn from the same universe as regards total caries experience in deciduous and permanent teeth and in eruption of permanent teeth and, hence, were combined into one single group for study.

Children in the study group are furnished a fluoridated water by the Washington Suburban Sanitary Commission although at the outset of the study one small group in Prince Georges County used water from wells. Raw water is taken from the Patuxent River and from the northwest branch of the Anacostia River. The two watersheds drain about 105 square miles to the north and east of the District of Columbia. The raw water receives similar treatment in two processing plants. Treatment includes aeration, prechlorination, flocculation, rapid sand filtration, adjustment of alkalinity with hydrated lime, postchlorination, and the addition of fluoride as sodium fluosilicate by means of dry feeders. With the exception of fluoridation these procedures have been uniform throughout the lives of the children under study. Tap water prior to 1952 was essentially fluoride free.

The fluoride feeders were started on December 28, 1951. During a period of preliminary adjustment, operation was not continuous. For this reason and because of the reservoir of fluoride-free water stored in the system of mains, a fully fluoridated water was not available at all taps throughout the distribution system until the last week of February in 1952. From that time onward, spot tap fluoride determinations carried out by the Washington Suburban Sanitary Commission have been faithful reflections of the daily fluoride levels in finished water at the filtration plants with the exception of a short period in mid-June 1952, when water in the Prince Georges County area was diluted with fluoride-free water obtained through a cross connection with the District of Columbia supply. During that period the fluoride level in the Suitland area dropped to 0.50 p.p.m. F. The District of Columbia supply has since been fluoridated. The average daily fluoride content of finished water at the Robert B. Morse filtration plant, serving generally the Montgomery County study children, was 0.98 p.p.m. F in 1952 and 0.94 p.p.m. F in 1953. The average daily fluoride content of finished water at the Patuxent filtration plant, serving generally the Prince Georges County study children, was 0.90 p.p.m. F over both years.

METHODS AND CRITERIA

All examinations have been carried out by dental officers of the National Institute of Dental Research. Mouth mirror and explorer are employed, with the child seated in a portable dental chair, under a portable examination light. Examination results are dictated to a recorder in a code similar to that suggested by Klein and Palmer (*6*). Some changes have been made in the code to avoid phonetic confusion and to permit the separate notation of pit-and-fissure or smooth-surface carious lesions found on a single tooth surface. Examinations are scheduled so that each child is observed during the same calendar week on successive years.

All critria are designed to assure the highest practicable degree of comparability among examiners:

None but positive lesions which admit the explorer point are recorded as carious.

Decalcified areas in the gingival third of the labial or buccal surfaces and opacities of marginal ridges which suggest a possible proximal lesion are recorded as questionable and tabulated as normal unless a definite enamel discontinuity is demonstrated with the explorer.

Deep pits and fissures are similarly recorded as questionable unless softened dentin is encountered by the explorer point or there is visible evidence of backward decay at the dentino-enamel junction.

A tooth is considered to be in eruption if any portion protrudes through the gum.

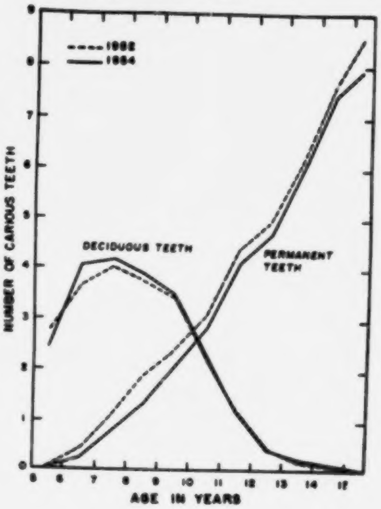
Criteria for the scoring of periodontal disease have been discussed in detail in an earlier report (*7*).

INITIAL STATUS

A total of 4,737 white children aged 5 through 15 years were examined between December 1951 and February 1952, coincident with the fluoridation of the water supply. The dental caries experience of this group of children is summarized in table 1.

Subsequent study of history records supplied by parents disclosed that 1,950 of these children had been born in and had been continuous residents of the area, except for short vacation trips in some instances, and had not received any treatment designed to prevent dental caries. Findings for this group, designated hereafter as the baseline population, were accepted as representative for children in the general area in the absence of a known caries-inhibitory factor and were utilized in the construction of a family of expectancy curves for caries increments in teeth and tooth surfaces, the eruption of permanent teeth, and the exuviation of deciduous teeth. Oral findings for these children are presented in broad outline in table 2.

1952 and 1954 caries experience of continuous-residence children of Montgomery and Prince Georges Counties, Md., who had not received caries prevention treatments.



The general caries experience in the baseline group is considerably lower than it was in children examined initially at Grand Rapids (*8*), and it was somewhat below the caries levels reported at the beginning of the Newburgh and Brantford studies (*9, 10*). Very similar findings were returned by Arnold and McClure (*11*) in a study of children aged 12 and 13 years in 1939 and 1940 in Arlington, Va., another suburb of Washington, D. C., and by the Southern Maryland District Dental Society, which examined about 15,000 other children in Montgomery and Prince Georges Counties early in 1952. The findings for this separate group of children are summarized in table 3.

Table 1.—*Oral status of 4,737 children in Montgomery and Prince Georges Counties, Md., 1952*

Mean age (years)	Number of childern	Mean number of permanent teeth		Mean number of deciduous teeth		Percent of caries-free children	
		In eruption	DMF[1]	Present	def[2]	Permanent dentition	Deciduous dentition
5.44	186	1.08	0.03	19.19	2.15	97.3	47.3
6.47	416	5.34	.30	16.22	3.16	86.1	33.7
7.45	487	9.14	.96	13.30	3.50	56.7	28.1
8.49	475	11.73	1.74	11.17	3.56	36.2	21.1
9.48	499	14.21	2.46	8.80	3.45	22.6	20.6
10.47	394	17.99	2.94	5.58	2.35	19.0	34.0
11.51	420	22.26	4.01	2.74	1.27	11.7	54.8
12.53	601	25.45	4.88	1.05	.52	9.0	75.7
13.50	572	27.09	6.29	.35	.19	7.2	89.0
14.49	513	27.53	7.86	.14	.05	3.9	95.9
15.37	174	27.67	8.63	.02	.02	4.0	98.3
All children—10.64	4,737	18.09	3.71	6.49	1.84	28.4	54.0

[1] Decayed, missing, or filled.

[2] Decayed, extraction indicated, or filled.

Table 2.—*Oral status of 1,950 continuous-residence children in Montgomery and Prince Georges Counties, Md., who, on initial examination in 1952, had not had topical fluoride or other caries-preventive treatments*

Mean age (years)	Number of children	Mean number of permanent teeth		Mean number of deciduous teeth		Percent of caries-free children	
		In eruption	DMF[1]	Present	def[2]	Permanent dentition	Deciduous dentition
5.44	60	1.40	0.03	19.10	2.77	98.3	36.7
6.46	171	5.63	.41	16.04	3.67	81.3	26.9
7.45	211	9.22	1.09	13.29	4.04	50.7	22.3
8.50	181	11.59	1.90	11.27	3.78	32.6	14.9
9.47	223	13.97	2.42	9.07	3.43	19.7	18.4
10.47	199	18.28	3.09	5.32	2.30	16.6	33.7
11.50	191	22.47	4.39	2.59	1.29	12.6	52.4
12.54	228	25.48	4.96	1.03	.47	8.3	77.2
13.51	233	27.05	6.15	.32	.16	6.9	91.0
14.50	188	27.56	7.66	.18	.07	3.7	94.1
15.36	65	27.80	8.57	.00	.00	7.7	100.0
All childern—10.60	1,950	18.07	3.69	6.49	2.03	26.3	50.3

[1] Decayed, missing, or filled.

[2] Decayed, extraction indicated, or filled.

Table 3.—*Mean numbers of decayed, missing, or filled permanent teeth reported for 14,936 white children in Montgomery and Prince Georges Counties, 1952*[1]

Age last birthday	Number of children examined	Mean number of DMF[2] teeth
5	409	0.04
6	1,360	.34
7	1,389	1.08
8	1,428	1.78
9	1,333	2.44
10	1,167	3.19
11	1,059	3.79
12	1,260	5.10
13	1,145	6.36
14	1,150	7.80
15	1,238	8.83
16	1,057	10.68
17	811	11.68
18	117	10.68
19	12	15.33
20	1	7.00

[1] From unpublished data reported by Southern Maryland District Dental Society to Public Health Service Region III (Washington, D.C.).

[2] Decayed, missing, or filled.

On the basis of these comparisons, the mean number of decayed, missing, or filled (DMF) permanent teeth in baseline children aged 11 years appears to be somewhat high. If this is true, computations of yearly caries increment based on these data will overestimate the true increment between the ages of 10 and 11 years and will underestimate the true increment between the ages of 11 and 12 years.

Table 4 shows crude caries rates for children, comparable with the baseline group, who were observed in 1952, 1953, and 1954. Findings for caries in permanent teeth in 1953, after 1 year of fluoridation, are in general slightly higher than the findings for the baseline year though the differences are well within the range of chance variation. This has been a common phenomenon in fluoridation studies where examiations are carried out with mirror and explorer. Reported DMF means were somewhat higher after 1 year of fluoridation at Grand Rapids and Brantford (*8, 10*). The effect is specifically graphed, for first molars, in the third year report from the Newburgh-Kingston study (*12*).

Lesions detected for the first time by mirror and explorer, after 1 year of fluoridation, are mostly lesions which began before fluoridation was instituted. By the end of the second year, however, it may be assumed that the majority of lesions detected for the first time by the explorer had their inception after fluoridation began. In the present study population, the second postfluoridation examination shows a drop in total numbers of decayed, filled, or missing permanent teeth, a drop averaging 0.27 teeth per child. Mean numbers of deciduous teeth which are decayed, filled, or indicated for extraction (def) are unchanged or slightly higher.

Mean caries data for the baseline children examined in 1952 and for comparable children examined in 1954 after 2 years of fluoridation are illustrated in the accompanying chart (see p. 231).

INCREMENTS OF NEW DMF TEETH

Expectancy increments of newly decayed, missing, or filled permanent teeth over a period of 1 year were computed for each age group, by the method used in the Hagerstown studies (*13*), from findings for the 1952 baseline group. Baseline children aged 7 years, for example, had an average of 1.090 decayed, missing, or filled permanent teeth. Six-year-old children had an average of 0.409 decayed, missing, or filled permanent teeth. The difference between these two findings, 0.681 teeth, was accepted as the expected average increase in carious permanent teeth as a group of children goes from the age of about 6½ to about 7½ years. Similar computations yielded the array of expected yearly caries increments shown in table 5.

Among comparable children examined in 1954, there were 1,218 aged 6 through 15 years who had also been examined in 1953. By matching 1953 and 1954 examination records for each of these children, actual numbers of newly carious permanent teeth were determined and analyzed as mean changes over the 12-month period. This method permitted the computation of standard error for each mean change and estimation of the

Table 4.—*Summary of findings for continuous-residence children in Montgomery and Prince Georges Counties, Md., who used city water, had not received topical fluoride or other caries-preventive treatments, and were examined in 1952, 1953, and 1954*

Age last birthday	Number of children examined			Mean number of DMF[1] teeth				Mean number of def[2] teeth			
	1952	1953	1954	1952	1953	1954	Difference, 1952 and 1954	1952	1953	1954	Difference, 1952 and 1954
5	60	67	94	0.03	0.02	0.01	−0.02	2.77	2.06	2.49	−0.28
6	171	238	306	.41	.32	.22	−.19	3.67	4.05	4.07	+.40
7	211	175	268	1.09	1.01	.80	−.29	4.04	3.95	4.19	+.15
8	181	192	189	1.90	1.63	1.37	−.53	3.78	3.93	3.91	+.13
9	223	175	192	2.42	2.43	2.08	−.34	3.43	3.36	3.52	+.09
10	199	210	190	3.09	3.01	2.86	−.23	2.30	2.41	2.33	+.03
11	191	181	204	4.39	3.82	4.11	−.28	1.29	1.11	1.25	−.04
12	228	245	279	4.96	5.13	4.70	−.26	.47	.68	.44	−.03
13	233	252	284	6.15	6.65	5.99	−.16	.16	.23	.23	+.07
14	188	287	249	7.66	8.35	7.43	−.23	.07	.07	.12	+.05
15	65	119	116	8.57	9.62	7.92	−.65	.00	.04	.03	+.03
All childern	1,950	2,141	2,371	3.69	4.11	3.42	−.27	2.03	1.91	2.08	+.05

[1] Decayed, missing, or filled.

[2] Decayed, extraction indicated, or filled.

probability that any observed increment varied only by chance from the absolute expectancies computed from the 1952 data. These analyses are also summarized in table 5.

If the presumptive 1952 increment rates had obtained throughout the year 1953–54, 1,130 newly carious permanent teeth would have developed in the group, or an average of 0.928 tooth per child. The actual increment was 910 newly carious permanent teeth, or an average of 0.747 per child—a difference of 19.5 percent. It is highly improbable that this difference of almost 20 percent is due to chance variation.

About the same result is obtained if expectancy increments are based upon the independent examination conducted by the Southern Maryland District Dental Society. From their data, 1,146 newly carious permanent teeth would have been predicted for the study children during the 1953–54 school year, a difference of 20.6 percent.

DISCUSSION

Comparisons of observed increments with expected increments, as computed from the 1952 baseline data, should be interpreted with caution. Each of the age groups in the 1952 data is an independent group, subject to biological variation. In the baseline array, DMF totals for children aged 11 years seem rather high. If this be true, the expected increment is an overestimate of DMF incidence between ages 10 and 11 and an underestimate of DMF incidence between ages 11 and 12. This factor of variation is present to a greater or lesser degree throughout the array. Little reliance, then, should be placed on the comparison of expected with observed increment in any specific age group. The study plan intends that age-specific incidence data shall ultimately be shown as trends, valid in themselves, with expectancy values serving only as points of departure.

On the other hand, age-specific error tends to be random, and overestimation at one age tends to cancel out underestimation at another if the entire group is considered as a unit. This is well illustrated by the close agreement between expectancy data calculated from the two independent examinations cited. No matter which one of these expectancy curves is used, the same conclusions are reached: that about 20 percent fewer permanent teeth became carious in the whole group than would have been expected on the basis of the 1952 examination and that it is highly improbable the difference is due to chance.

This particular method is not appropriate at all when applied to deciduous teeth without any means of determining whether a missing deciduous tooth has been lost prematurely or normally. On the basis of the cumulative totals shown in table 4, no change in the prevalence of caries in deciduous teeth is apparent in these study groups. Deciduous teeth in these children had been in eruption and at risk of caries for 3 years or more at the time the water supply was fluoridated.

SUMMARY

This preliminary report has described the examination criteria for dental caries and methods in use and has defined the population under observation in a continuing study of oral health in elementary and junior high school children of Montgomery and Prince Georges Counties, Md. The two counties are adjacent to the District of Columbia. The domestic water used by these children was fluoridated with sodium fluosilicate late in December of 1951. Evidence is presented that there was a statistically valid decrease of roughly 20 percent in the number of permanent teeth becoming carious during the second year of fluoridation, judged by findings from the baseline examination early in 1952.

Table 5.—*Increments of new decayed, missing, or filled permanent teeth over 12 months, from 1953 to 1954, in continuous residents of Montgomery and Prince Georges Counties, Md., who were examined in both years, used city water, and had not received topical fluoride or other caries-preventive treatments, as compared with increments computed from data for comparable children of the same counties in 1952*

Expected mean DMF [1] increment past year	Children examined in 1953 and again in 1954				
	Mean age, 1954	Number examined	Actual mean DMF [1] increment, 1953–54	Difference between actual and expected increments	Probability difference due to chance [2]
0.376	6.63	27	0.111± 0.082	−0.265	<0.001
0.681	7.51	160	.425± .070	− .256	< .001
0.811	8.45	121	.736± .102	− .075	.23
0.516	9.47	137	.489± .097	− .027	.39
0.668	10.51	120	.758± .120	+ .090	.23
1.302	11.45	133	1.075± .132	− .227	.04
0.569	12.47	106	.840± .156	+ .271	.04
1.194	13.53	175	1.006± .151	− .188	.11
1.510	14.49	171	.947± .176	− .563	< .001
0.909	15.39	68	.324± [illegible]	− .585	.01
0.928		1,218	.747± .046	− .181	< .001

[1] Decayed, missing, or filled.

[2] Based on areas under the normal curve.

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GRAND RAPIDS FLUORIDATION STUDY—RESULTS PERTAINING TO THE 11TH YEAR OF FLUORIDATION

Francis A. Arnold, Jr.

The control of dental caries by water fluoridation has been the subject of articles and reports for more than a decade. Relatively complete bibiographies on this subject are included in previous review dissertations. (*1–4*). It may be stated that all recognized scientific studies have demonstrated the certainty of this method as a public health procedure for securing better dental health for a community.

Although many of the studies on water fluoridation have been in progress long enough to evaluate the soundness of the procedure, much is to be learned concerning its various public health aspects. The purpose of the present paper is to review briefly the results of 10 years of one of these research projects; to summarize the findings of the eleventh year of the study, particularly those observations which pertain to dental health programs; and to evaluate the findings in respect to the results of other independent investigations of similar character. For this reason the results of three of these studies covering the first 10 years of operation will be discussed.

STUDY PLAN

Generally speaking, all of the studies referred to are designed to evaluate what, if any, effect fluoridation of a public water supply will have on the general health of the community using this supply. The public health responsibility has been foremost in the minds of all people concerned and has been recognized by everyone involved in initiating or endorsing such programs. The technical methods of evaluation used in these different research projects have been described previously (*5–8*).

Particularly, the study plans were designed to determine the prevalence of dental caries in a community prior to fluoridation and to evaluate the effects by subsequent annual oral examinations. In each of these studies due attention has been paid to determining whether fluoridation has any effect upon the general health, in addition to the dental health, of the population using the fluoride water.

The Grand Rapids study, for example, was designed to continue 10–15 years. For base line data all children in attendance in schools were examined in 1944, prior to fluoridation. In each succeeding year, an increasing number of selected age group samples of school children were examined. In 1954, after 10 years of fluoridation in Grand Rapids, the age distribution of the examined sample was relatively complete for the ages five through 16 (*9*). After the tenth year of observation, the study design called for a concentration of effort on a selected group of children more than 10 years of age (table 1). Plans have been made to continue examinations for these age groups in Grand Rapids until the observations cover persons who have been continuously exposed to fluoride water throughout their elementary and secondary school lives.

The study design also was altered to take into account the fact that Muskegon, the original control city, had inaugurated fluoridation in July, 1951. For this reason observations are being continued on a sample (a single school group) of children from this city mainly for the purpose of evaluating periodontal conditions. The results of these observations constitute a portion of a later report by Dr. A. L. Russell.

RESULTS

In summary form the results of 10 years of fluoridation in Grand Rapids are shown in table 2, both for deciduous and permanent teeth. In making comparisons on these data it should be remembered that Muskegon started fluoridation in July 1951. A general comparison of the results in Grand Rapids with those of two other studies (*10–11*), on the basis of 10 years' operation, is shown in table 3.

From a public health program standpoint the success of the procedure may be evaluated in part by considering the unmet needs of the population observed. In this respect two major factors of the DMF rate can be utilized to measure the potentiality or effectiveness of fluoridation as a caries control method; namely, the change in ratio of filled to unfilled teeth and the number of lost teeth. Using the data on the permanent teeth as presented in table 2, the changes in ratios of these factors are presented graphically in figures 1 and 2.

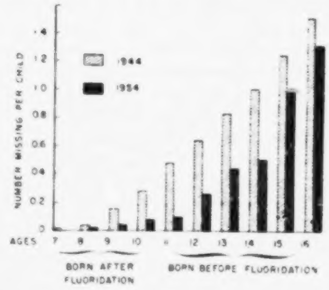

FIGURE 1.—*Missing permanent teeth per child before and after ten years of fluoridation (Grand Rapids, Mich.).*

As stated earlier, the annual examinations in the Grand Rapids study have continued through the eleventh year.

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* This paper was presented before a Joint Session of the Dental Health and School Health sections of the American Public Health Association at the Eighty-Fourth Annual Meeting in Atlantic City, N.J., Nov. 13, 1956.

TABLE 1.—*Distribution of continuous resident children examined in Grand Rapids, Muskegon, Mich., and Aurora, Ill., according to age and year of examination*

Age [1]	Aurora, Ill. 1945	Basic Examination 1944–1945	Grand Rapids, Mich.										
			1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955
4	30	323	540	300	168	137	75	117	168	116	101	77	—
5	407	1,633	1,714	831	886	842	777	720	853	1,087	715	529	—
6	473	1,789	1,186	628	663	736	697	748	750	826	1,010	561	—
7	516	1,806	149	82	69	55	54	438	423	422	410	751	—
8	469	1,647	15	216	135	138	155	501	470	444	390	567	—
9	368	1,639	—	525	465	484	519	520	582	720	623	477	—
10	397	1,626	—	109	108	111	125	131	141	512	499	515	—
11	383	1,556	—	17	18	22	140	130	151	246	291	499	27
12	401	1,685	174	85	38	60	130	200	176	211	316	260	168
13	401	1,668	953	547	625	600	574	530	497	497	557	224	254
14	433	1,690	273	173	196	152	153	130	128	119	111	250	254
15	467	1,511	80	53	80	64	64	58	53	80	99	240	307
16	371	1,107	4	3	233	245	209	177	198	191	197	198	212
	5,116	19,680	5,088	3,569	3,684	3,646	3,672	4,400	4,590	5,471	5,319	5,148	1,222
			Muskegon, Mich.[2]										
4		20		43	18	26	51	41	63	52	43	40	—
5		402		321	348	422	340	359	351	487	370	381	55
6		462		339	312	305	393	310	294	353	397	386	72
7		408		36	42	36	30	274	223	246	209	292	78
8		376		18	13	10	12	190	275	205	212	244	75
9		357		213	215	199	197	227	277	348	258	275	65
10		359		62	57	52	52	51	62	287	311	226	63
11		293		12	10	14	146	141	139	133	175	208	54
12		328		21	19	11	28	43	48	46	163	183	93
13		377		197	207	208	214	173	225	178	228	243	58
14		369		77	50	79	66	63	59	54	51	121	—
15		292		18	44	41	34	35	21	30	35	139	—
16		248		1	199	205	132	146	155	132	161	185	—
		4,291		1,358	1,534	1,608	1,695	2,053	2,192	2,551	2,613	2,923	613

1. See reference 4 relative to effect of selection of sample by grades on specific age groups.
2. The basic examinations in Muskegon were not done until late spring of 1945; therefore, no examinations were made in the fall of 1945.

The results of these observations on the 11- to 16-year age groups are shown as table 4, and indicate that the beneficial effects of fluoridation have continued on through the eleventh year of research.

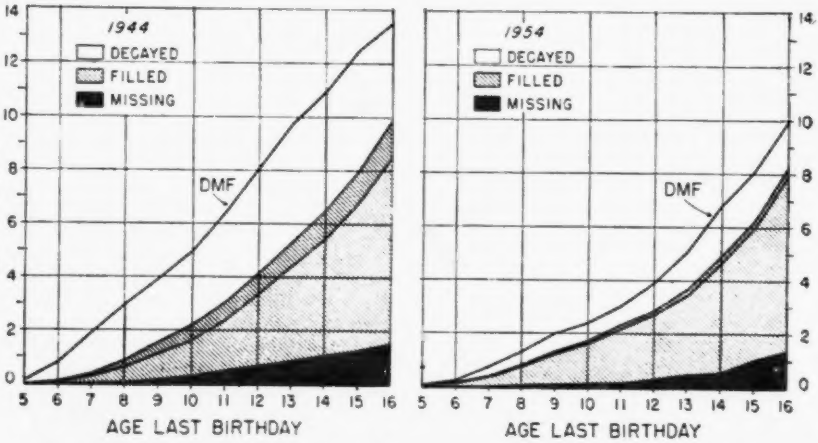

FIGURE 2.—*Dental caries prevalence in permanent teeth examinations made in 1944 and 1954 (Grand Rapids, Mich., ages 5–16).*

Prior to the inauguration of all of these studies the scientific evidence indicated that the fluoridation of water supplies would not produce a public health problem of dental fluorosis. It should be emphasized that full evaluation of the effects of fluoridated waters, as measured by dental fluorosis, are evaluated on the basis of erupted permanent teeth. The eleventh year of the Grand Rapids study gives a preliminary indication of the results to be expected. In table 5 the results in respect to dental fluorosis on the 11- to 16-year-old children are shown.

DISCUSSION

On the basis of these results and the data included in previous reports, one can conclude that fluoridation of public water supplies will effectively reduce the dental caries experience of those persons exposed to its effect continuously from birth onward. Also, sound scientific evidence shows that these beneficial results are not confined solely to those born subsequent to the inauguration of fluoridation. For example, the results shown for the 16-year age group in table 2 indicate a reduction of 26 percent

Table 2.—*Dental caries experience in deciduous and permanent teeth of continuous resident children of Grand Rapids and Muskegon, Mich., as observed in 1954 examination (from Pub. Health Rep. 71: 652 (July), 1956)*

Age Last Birthday	Deciduous Teeth		Permanent Teeth				Per cent of Caries-Free Children [4]
	Number Filled	Total def [1]	Decayed	Missing [2]	Filled	Total DMF [3]	
	Grand Rapids, Mich. Number of Teeth per Child						
4	0.68	2.12	——	——	——	——	——
5	1.14	2.50	0.01	——	0.01	0.02	99.4
6	1.30	2.95	0.11	——	0.09	0.19	89.3
7	1.45	3.26	0.36	——	0.35	0.69	66.8
8	1.42	3.31	0.52	0.02	0.77	1.27	49.4
9	1.30	3.00	0.74	0.04	1.26	1.97	33.1
10	0.98	2.35	0.73	0.07	1.63	2.34	26.6
11	0.63	1.32	0.78	0.10	2.19	2.98	16.8
12	0.12	0.44	1.14	0.26	2.55	3.87	13.5
13	0.04	0.18	1.56	0.44	3.23	5.05	10.7
14	——	——	2.13	0.52	4.36	6.78	5.6
15	——	——	2.08	1.02	5.23	8.07	1.2
16	——	——	1.96	1.35	6.90	9.95	2.0
	Muskegon, Mich.[5] Number of Teeth per Child						
4	1.18	3.03	——	——	——	——	——
5	0.98	3.98	0.03	——	——	0.03	98.4
6	1.64	4.85	0.33	0.00	0.13	0.45	79.8
7	1.96	5.35	0.74	——	0.41	1.14	49.7
8	2.03	4.98	1.23	0.06	0.95	2.18	27.5
9	1.60	3.81	1.29	0.14	1.80	3.16	14.5
10	1.14	2.75	1.44	0.23	2.20	3.72	5.7
11	0.44	1.42	1.79	0.32	2.67	4.58	4.3
12	0.14	0.61	2.05	0.42	3.85	6.12	4.4
13	0.06	0.12	2.47	0.72	4.97	7.98	1.6
14	——	——	4.31	1.39	5.31	10.74	——
15	——	——	3.55	1.42	6.51	11.19	1.4
16	——	——	2.78	1.42	8.77	12.55	1.1

1. Decayed, extractions indicated, or filled deciduous teeth.
2. Includes teeth listed as "remaining roots" and teeth destroyed beyond any possible repair.
3. Decayed, missing, or filled permanent teeth; each tooth is counted only once for this total. A tooth which has both a filled and a carious surface will be included under both the "decayed" and the "filled" column.
4. Permanent teeth only.
5. It should be noted that these children had already received the beneficial effects of 3 years of water fluoridation (see text).

in the expected amount of caries. The results of the two other studies referred to in table 3 are similar to this observation.

A similar analysis of the data can be carried back into the results of previous reports on these studies, making comparisons on the basis of annual observations. Such an analysis suggests that the "caries susceptibility" of a tooth may be affected by water fluoridation even after the tooth has been formed and erupted. This hypothesis is more completely discussed and evaluated in another report (*12*). The evidence from the studies reviewed in this presentation indicates that fluoridation has a beneficial effect on teeth which are not fully formed, or are not fully maturated in the oral environment.

From the public health standpoint there necessarily stems an interest in the practicability of the procedure. No attempt will be made to evaluate the economics of the technical aspects involved in water fluoridation other than to say that the process is comparatively inexpensive with only a rare exception. As far as dental health is concerned, there can be no doubt from the data shown in figures 1 and 2 that the dental health problem in Grand Rapids, for example, has been brought under a better measure of control. If considered only in terms of dollars and cents, the savings to this community have been tremendous over the past 11 years. Economic considerations of this character, however, are usually misleading as far as evaluating public health procedures are concerned.

As stated earlier, the effects of water fluoridation on public health from aspects other than dental caries were fully considered before the inauguration of any of these programs. On the basis of previous scientific evidence investigators anticipated that water fluoridation would produce a slight increase in the prevalence of the mildest forms of dental fluorosis. Although sufficient time has not elapsed to evaluate this factor fully, the results to date (table 5) show that the degree of fluorosis produced by fluoridation at levels recommended for caries control is of no public health significance.

In all these studies particular attention and interest has been paid to any effects fluoridation might have on other biologic systems of the body. In none of the studies has there been any scientific evidence to suggest an adverse effect on any segment of a rather large population living under divergent environmental conditions. These observations, in conjunction with the fact that millions of people have used naturally fluoridated waters for generations, attest to the complete safety of the procedure.

SUMMARY

The observations of the Grand Rapids study for the past 11 years of water fluoridation have been summarized. The results of this study, together with others which have been conducted for similar periods, indicate the feasibility of this procedure for the control of dental caries. In all studies the findings show a reduction of 60–65 percent in the prevalence of caries in the permanent teeth of children born subsequent to the change in water supply. Furthermore, the evidence strongly suggests beneficial effects on teeth which were formed, or erupted, prior to the initiation of water fluoridation.

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Table 3.—*General comparison of dental caries experience rates for deciduous (def) and permanent (DMF) teeth after 10 years of fluoridation in Grand Rapids, Mich., Brantford, Ont., and Newburgh, N.Y.*

(Data from References 9–11)

Age (Last Birthday)	Grand Rapids, Mich. No. Children Examined		Grand Rapids, Mich. Per cent Reduction Caries Rate		Brantford, Ont. No. Children Examined		Brantford, Ont. Per cent Reduction Caries Rate		Newburgh, N. Y.[1] No. Children Examined	Newburgh, N. Y.[1] Per cent Reduction Caries Rate
	1944	1954	def	DMF	1944	1955	def	DMF	1954 1955	DMF
6	1,789	561	54	75	556	485	57	60		
7	1,806	751	48	63	616	448	52	67	708	58
8	1,647	567	43	57	614	483	43	54	(6–9 yrs.)	
9	1,639	477	35	50	608	423	34	46		
10	1,626	515	17	52	565	301	33	41		
11	1,556	499	2	54	604	280	−2	39	521	53
12	1,685	260	—	52	658	336	—	48	(10–12 yrs.)	
13	1,668	224	—	48	561	247	—	42	263	48
14	1,690	250	—	38	307	93	—	36	(13–14)	
15	1,511	240	—	35	105	37	—	35	—	—
16	1,107	198	—	26					109	41

1. Results based on difference between Newburgh and Kingston after 10 years fluoridation and include both clinical and roentgenographic findings.

Table 4.—*Dental caries prevalence in permanent teeth of continuous resident Grand Rapids children as observed in 1955 (11 years after fluoridation)*

Age	No. Examined	No. DMF Teeth per Child	Carious Teeth per Child	Filled Teeth per Child	Missing Teeth per Child	Per cent Reduction in DMF Teeth (1944–1955)
11	27	2.85	0.93	2.07	0.04	55.5
12	168	3.36	0.85	2.55	0.10	58.3
13	254	4.86	1.41	3.10	0.45	50.1
14	254	6.13	1.65	4.03	0.60	44.0
15	307	7.86	2.07	5.24	0.80	37.1
16	212	8.93	1.90	6.32	0.93	33.9

Table 5.—*Amount and degree of dental fluorosis observed in Grand Rapids, Mich.. after 11 years of fluoridation*

Age	No. Children Examined	Fluorosis Classification[1] Normal		Questionable		Very Mild		Mild	
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
11	27	24	89	2	7	1	4	0	
12	168	155	92	8	5	5	3	0	
13	254	243	96	8	3	3	1	0	
14	254	243	96	9	4	2	1	0	
15	307	302	98	4	1	1	0.3	0	
16	212	209	99	2	1	1	0.5	0	

1. Classification according to Dean, reference 2.

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DETERMINING OPTIMUM FLUORIDE CONCENTRATIONS

DONALD J. GALAGAN and JACK R. VERMILLION

The fluid intake study among children in Antioch and Brentwood, Calif., in 1953–54 provides a basis for determining the optimum fluoride concentration in water supplies in relation to environmental temperature. This study showed that for every degree increase in maximum daily temperature between 50° and 100° F. water intake increased, on the average, by 0.062 ounces per pound of body weight. For example, the average daily water consumption per pound of body weight was 0.272 ounces when the maximum daily temperature was 50° F. and 0.334 ounces when the maximum daily temperature was 60° F.

The relationship between maximum temperature and water intake for the California children was described by the estimation equation "ounces of water per pound of body weight= —0.038+0.0062 temperature." The validity of this equation should perhaps be checked by studies in other areas of the country, but in the meantime it can be used to illustrate the calculation of optimum fluoride concentrations. As will be pointed out later in the paper, results obtained with the equation in the Chicago area, where optimum fluoride concentration is known from epidemiological studies, indicate that it is reasonably reliable.

The basic structure of the formula developed for estimating optimum fluoride concentration is: parts per million of fluoride=optimum water consumption÷estimated water consumption. Thus, the optimum fluoride concentration for a given community is equal to a constant (the average amount of water containing 1 p.p.m. fluoride that affords optimum protection against dental caries) divided by the estimated water consumption of children in a given community. Both measures are in ounces of water consumed daily per pound of body weight.

If daily maximum temperature data for the Chicago area are applied to the water estimation equation, it is possible to calculate a denominator value for the formula, that is, the average amount of water that would be consumed daily per pound of body weight by children in the Chicago area aged 10 years or less. The number derived, although it has no particular meaning in itself, may also serve as the constant for the formula, since it is known from epidemiological data that the optimum fluoride concentration for the Chicago area is 1 p.p.m. The constant, or numerator, in the formula must equal the estimated water consumption, or denominator, for the optimum fluoride concentration to be 1 p.p.m.

The estimated water consumption for the Chicago area children is based on weather data for two towns, Maywood and Joliet. Since both of these towns were included in the early studies of Dean (*1*), the fluoride concentration and the fluorosis index are known. Although both communities have more than 1 p.p.m. of fluoride in their water supplies, 1 p.p.m. is considered the optimum concentration.

TABLE 1.—*Mean maximum temperatures and calculated optimum fluoride concentrations for selected communities in the United States*

Community	Mean maximum temperature [1]	Calculated optimum fluoride concentration
Arizona:		
Chandler Heights	85.3	0.7
Tucson	82.6	.7
California:		
Los Angeles	73.9	.8
San Francisco	61.7	1.0
District of Columbia	68.7	.9
Illinois:		
Joliet	61.6	1.0
Maywood	61.8	1.0
Louisiana:		
New Orleans	78.7	[illegible]
Shreveport	77.7	[illegible]
Montana:		
Billings	59.1	1.0
Butte	51.7	1.2
North Carolina:		
Charlotte	72.7	.8
Rocky Mount	73.2	.8

[1] Based on temperature data for the 5-year period 1951–55 from U. S. Weather Bureau publications entitled "Climatological Data."

The estimated average water consumption, the denominator of the formula, which is hereafter called *E*, is calculated by obtaining the mean maximum tempearture for at least a 5-year period for the community in question and substituting this figure in the estimation equation *E*= —0.038+0.0062 temperature. The resulting value represents the average number of ounces of water per pound of body weight that children through 10 years of age would be expected to drink daily under the temperature conditions of the community. The *E* value for both Maywood and Joliet is 0.34, and, as explained previously, this number may serve as the constant for the formula.

The value 0.34 for the constant is reasonable from the standpoint of optimum fluoride ingestion through water. For example, again in terms of average phenomena, 0.34 ounces of water fluoridated at 1 p.p.m. contains approximately 0.011 mg. of fluoride. In the fluid intake study in California, the average body weight of children studied was about 46.5 pounds. At this weight, the children ingest about 0.5 mg. of fluoride daily through their drinking water. This amount is similar to other estimates of the amount of fluoride that should be ingested through water for optimum dental health (*2*). This fact tends to strengthen confidence in both the constant and the water estimation equation from which it was derived.

With the formula "parts per million of fluoride=0.34÷*E*," optimum fluoride concentrations have been calculated for selected communities representing various geographic areas throughout the United States. The results are shown in table 1. For the cities listed, the lowest concentration, 0.7, is suggested for the Arizona communities; the highest, 1.2, for Butte, Mont.

To obtain a fluoride concentration lower than 0.7 p.p.m. with this formula, the mean maximum temperature would have to average at least 90.6° F. To obtain one greater than 1.2 p.p.m., the mean maximum temperature would have to be lower than 50° F. The possibility of the occurrence of either of these extremes in the United States seems remote. Consequently, it is not expected that the optimum concentration for any community would be outside the range 0.7—1.2 p.p.m. generally recommended for fluoridation.

In the practical application of the method described here for determining optimum fluoride concentrations, it is not necessary to make calculations for each community. Optimum fluoride levels for ranges of mean maximum temperatures from 50° through 90.5° F. are presented in table 2. The optimum fluoride concentration for a community may be determined simply by obtaining the mean maximum temperature for a 5-year or longer period from appropriate publications of the United States Weather Bureau and then referring to this table.

Two points must be stressed in the application of this method. First, the temperature figure should be at least a 5-year average for the figure to be truly representative. Second, the mean maximum temperature in degrees Fahrenheit

Table 2.—*Mean maximum temperatures and corresponding recommended optimum fluoride concentrations*

Mean maximum temperature (degrees Fahrenheit)	Recommended optimum fluoride concentration (parts per million)
50. 0–53. 7	1. 2
53. 8–58. 3	1. 1
58. 4–63. 8	1. 0
63. 9–70. 6	. 9
70. 7–79. 2	. 8
79. 3–90. 5	. 7

is the only measure that can be used since the method is based on an equation for estimating water consumption in which this measure is used.

SUMMARY

The following formula, which takes into account variations in environmental temperature, is suggested for determining optimum fluoride concentrations for community water supplies:

$$\text{parts per million of fluoride} = \frac{0.34}{E}$$

The figure 0.34 (optimum water consumption) was calculated from data for an area where the optimum fluoride concentration is known. E is the estimated average daily water intake for children through 10 years of age in ounces per pound of body weight. It may be calculated from the estimation equation $E = -0.038 + 0.0062$ temperature, where temperature is the mean maximum temperature in degrees Fahrenheit.

A table showing suggested optimum fluoride concentrations for communities with 5-year mean maximum temperatures of 50° through 90.5° F. is presented.

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POSTERUPTIVE EFFECTS OF FLUORIDATION ON FIRST PERMANENT MOLARS OF CHILDREN IN GRAND RAPIDS, MICHIGAN

Richard L. Hayes, Norman W. Littleton, and Carl L. White

The discovery that there was less dental caries among children who had ingested fluoride-bearing water all their lives (*1*) led to the initiation in 1945 of several fluoridation studies (*2–4*). These were designed to learn whether or not artificial fluoridation would also reduce the incidence of caries in children using fluoride from birth. It was apparent, from the reports of these (*2, 4, 5*) and other studies (*6, 7*) that caries was also inhibited in children who had ingested fluoridated water sometime during the period prior to the eruption of the permanent teeth. Not only did these preeruptive effects appear earlier than anticipated, but the data also suggested that there was a post eruptive inhibition of caries in first permanent molars (*4*).

There is other evidence which shows that fluoride will inhibit caries when it is administered after the eruption of the permanent dentition. An early laboratory finding (*8*), confirmed by other investigators (*9–11*), demonstrated that rat molar teeth could be protected by fluoride given after the eruption of the teeth. In human beings posteruptive effects have been reported for natural fluoride-bearing waters (*12–16*) and for a topically applied fluoride solution (*17*).

The present study is specifically concerned with determining whether or not artificial fluoridation will have any long-term caries inhibitory effect on human teeth already erupted when the water supply was fluoridated. This question has not been answered by any of the reports cited, as the studies dealing with posteruptive effects were of short duration, and those of long duration did not separate the posteruptive from the preeruptive effects.

The Grand Rapids fluoridation study (*2, 19, 20*) offered an opportunity to investigate the posteruptive effects of water fluoridation. In Grand Rapids there were children whose first permanent molars had erupted before fluoridation began and who had used fluoridated water since January, 1945, or about 11 years. In Muskegon, there were similar children who had used fluoridated water since July, 1951. The study situation was not ideal because the Muskegon children had been ingesting fluoridated water for nearly five years. However, those children who had first permanent molars in eruption in 1945 would have been at least 12 years of age in 1951. There is evidence (*15*) that posteruptive use of water containing as much as 3 p.p.m. of natural fluoride does not affect the number of decayed, missing, or filled erupted first permanent molars of children 12 years of age or older but approximal surfaces, which continue to decay after 12 years of age (*18*), might be affected. In any event, comparison with Muskegon findings should result in a conservative estimate of the posteruptive effect in Grand Rapids.

SELECTION OF CHILDREN

From the 1944–1945 examination records of public school children who had then resided continuously in their respective cities since birth, the authors selected all of the first-grade children who had two or more first permanent molars in eruption at the time of the prefluoridation examinations. The directories of the two cities were consulted to identify the children whose families still resided in the same city. At the time of the examinations in April 1956, 211 were enrolled in the five Grand Rapids public high schools and 141 in the Muskegon public high school. Of these, 196 were examined in Grand Rapids and 138 in Muskegon. For further retention in the study the Grand Rapids children were required to have used water fluoridated to a level of 1 p.p.m. from January 1945, to the date of examination in April, 1956. Muskegon children were required to have used no fluoride-bearing water prior to July 1951, and no water containing fluoride in excess of 1 p.p.m. since July 1951.

Vacation trips of 90 days or less were not considered. In all, 184 Grand Rapids and 133 Muskegon children met these requirements and were retained as the total study group.

PROCEDURE

The clinical examinations were performed with a mouth mirror, SSW No. 3 DE explorer and artificial illumination with a portable Castle T-V5A lamp. The first permanent molars were given a detailed examination and the size of restorations and cavities were schematically recorded by the examiner on diagrams of the first permanent molars.

Four sizes of first permanent molar lesions were recorded:

1. Small: A pit in which the explorer would catch, but could not be rocked; a restoration 1.5 mm. or less in width; and smooth surface caries which could be so restored. An approximal cavity was recorded as small only if there was no approximating tooth.
2. Medium: A pit or fissure in which the explorer could be rocked, but which could not be diagnosed without use of the explorer; restorations resembling the ideal in size and shape; and smooth surface lesions which could be so restored, including approximal explorer catches.
3. Large: All cavities which could be diagnosed without use of the explorer, and restorations appreciably larger, in any dimension, than the ideal.
4. Pulp exposures: Cavities which, in the opinion of the examiner, involved the pulp. The explorer was not used on such cavities.

During the examinations models of typical small, medium, and large restorations were used for reference. If there was any doubt about the first molar being missing, the second molar was examined.

Posterior bite-wing x-rays were taken with a 5 ma 45 Kvp portable Oralix dental X-ray machine to which 1 mm. of aluminum filtration had been added. At least four films were used for each person; more, including periapicals, were used for difficult cases. The angulation of the X-ray beam was varied as required by the alignment and shape of individual first molars. The Kodak DF-58 films were exposed for 1½ seconds and developed for 3 minutes at 68° F.

After the X-rays from the two cities were randomized so that the reader did not know whether a given set of films was from Grand Rapids or Muskegon, the interpretations were made in two stages. First, the films were read for the sole purpose of identifying the first permanent molars. If there was any doubt about the presence or absence of a first permanent molar the X-rays were discussed with the other investigators and a decision reached by mutual agreement. These data were used to modify the clinical examinations. The films were then read for approximal caries of first permanent molars. Cavities and restorations were recorded as one of four depths: enamel only, shallow, moderate, or deep dentin. The classification was based upon the deepest penetration of caries or the deepest portion of the approximal restoration. For dentinal caries the distance from the dentino-enamel junction to the nearest point of the pulp was divided into thirds.

The value of this information about the size of the cavities was limited, unless it could be used to yield a single number that would express the caries experience of each individual. This number could then be used to compute group means and error terms. Borrowing the technics of other workers (*21–24*) the authors assigned scores to the clinical findings: a score of one was assigned to a small cavity or restoration, a score of two to a medium cavity or restoration, a score of three to a large cavity or restoration, and a score of four to a pulp exposure or a missing surface. The scores for each individual were totaled and divided by the number of surfaces at risk, i.e., the number of first molar surfaces present and caries-free in 1944–1945. The X-ray findings of enamel, shallow dentin, moderate dentin, and deep dentin were given scores of one, two, three and four, respectively. Missing approximal surfaces were also given a score of four. The X-ray scores for each individual were totaled and divided by the number of approximal surfaces at risk. When the clinical and X-ray findings were combined, the larger of the two scores for a particular approximal surface was used. For convenience, the term "caries-score" will refer to the quotient:

$$\frac{\text{Sum of the scores for one individual}}{\text{Number of tooth surfaces at risk}}$$

"Mean caries-score" will, of course, signify a group score.

STUDY PROBLEMS

The caries experience of all the 6-year-old children in these cities was almost identical in 1944–1945 (*2*). The manner of drawing our samples, however, did not assure that our study groups would be representative of the whole six-year-old population examined in 1944–1945. The sample actually assembled from the first grade proved to be dissimilar with respect to caries experience in 1944–1945. Our Grand Rapids total study group had in 1944–1945 an average of 1.05 decayed, missing, or filled permanent teeth as compared with an average of 1.8 for our Muskegon group.

The original difference in caries susceptibility presented a problem in

TABLE 1.—*Comparison of the study groups: Mean age, eruption and caries-status according to the 1944–1945 clinical examinations, Grand Rapids and Muskegon, Mich.*

	Matched Study Group		Unmatched Group		Total Study Group	
	Grand Rapids	Muskegon	Grand Rapids	Muskegon	Grand Rapids	Muskegon
Number of children:	116	116	68	17	184	133
Males	51	51	42	8	93	59
Females	65	65	26	9	91	74
Mean age (years)	6.46	6.65	6.62	6.02	6.52	6.57
First permanent molars:						
Number erupted	410	413	240	58	650	471
Mean number erupted	3.53	3.56	3.53	3.40	3.53	3.54
Number decayed, missing, or filled (DMF)	136	137	56	43	192	180
Adjusted mean number DMF *	1.17	1.17	0.82	2.62	1.05	1.35
Number of caries-free surfaces	1,884	1,882	1,130	227	3,014	2,109
Number of decayed or filled (DF) surfaces	166	183	70	63	236	246
Adjusted mean number DF surfaces *	1.43	1.57	1.03	3.83	1.28	1.85
Deciduous molars and canines:						
Number decayed, missing, or filled	770	771	272	158	1,042	929
Mean No. decayed, missing, or filled	6.64	6.65	4.00	9.29	5.66	6.98

* Adjusted to the number of teeth in eruption in Grand Rapids.

analysis as the long-term, posteruptive effect was not expected to be large enough to overwhelm this difference. Therefore, a matching process was used to select groups with the same initial caries experience. For each sex and each city the 1944–1945 examinations were separated into subgroups having the same total caries experience: the number of decayed, missing, or filled permanent teeth, plus the number of decayed, missing, or filled deciduous canines and molars. Examinations from these subgroups were matched for first permanent molars in eruption, number of first permanent molars with caries and, when possible, for location and severity of caries. Perfect matches were not possible in all instances, but care was taken to assure that the totals for the matched groups were similar.

The matching process, by excluding the older Grand Rapids children with low caries experience, resulted in two similar groups of 116 children each. The characteristics of the matched study groups are compared with the total study group and with the unmatched group in table 1. In the matched group there were differences (not statistically significant) in mean age (about 2½ months) and in the number of decayed or filled surfaces of first permanent molars. These differences might be related.

A second study problem was the identification of first permanent molars, which is not a new problem (*25, 26*). In the total study group, when all first permanent molars (1,268) were used regardless of their 1944–1945 eruption status, there were 43 disagreements (3.4 percent of all first molars) between the clinical and X-ray identification. There was reason to believe that the clinical examiner was correct for some of the disagreements, but the X-ray identification was accepted for the purpose of this study.

A third study problem was the comparability of the clinical and X-ray judgments of the size of restorations. This subject could not be directly examined since the two methods estimate different aspects of size: the clinical examination estimates size by surface area, the X-ray interpretation by depth. However, the ratio of depth (X-ray) to area (clinical) was 1.34 in Grand Rapids and 1.30 in Muskegon. The small difference in these ratios is not considered important.

RESULTS AND INTERPRETATION

The data from the randomized X-rays indicate that fluoride does inhibit caries on the approximal surfaces of first molars erupted prior to fluoridation. Thus in the matched groups, there were more normal approximal surfaces present in Grand Rapids than in Muskegon, and the caries which was present was shallower in Grand Rapids. These findings are shown in figure 1, which compares the approximal caries that occurred in the Grand Rapids matched group between 1944–1945 and 1956 with the approximal caries which occurred in Muskegon. These data present the clearest picture of the contrast between the two cities, but are difficult to subject to a statistical test because the surface, rather than the person, is the unit.

The data from the usual technic of adding the clinical and X-ray findings also suggest that fluoride confers a posteruptive protection on first permanent molars. In the Grand Rapids matched group about 10 percent fewer surfaces became decayed, missing, or filled between 1944–1945 and 1956 than in Muskegon. These data are presented in table 2, which also includes the incremental caries experience of the unmatched children and of the total study group.

When the missing surface component is ignored, and the data from the matched group analyzed according to surface type (table 3) it appears that there was a protective effect on all surfaces except the occlusal. Failure of the occlusal surface to derive benefit may be an artifact due to the placement of prophylactic restorations or to the restoration of this surface in the process of restoring approximal surfaces.

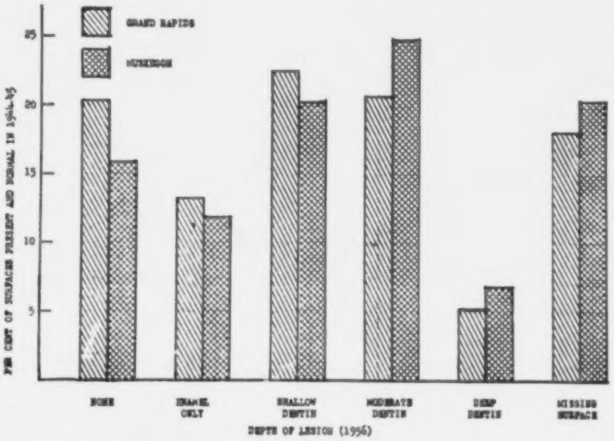

FIGURE 1.—*Comparison of approximal caries occurring between 1944–1945 and 1956 in first permanent molars erupted in 1944–1945, Grand Rapids and Muskegon matched groups, X-ray interpretations only*

TABLE 2.—*Comparison of incremental caries occurring between 1944–1945 and 1956 in first permanent molars erupted in 1944–1945, Grand Rapids and Muskegon matched, unmatched, and total groups, clinical examination plus X-ray lesions of dentin*

	Matched Study Group		Unmatched Group		Total Study Group	
	Grand Rapids	Muskegon	Grand Rapids	Muskegon	Grand Rapids	Muskegon
Number of new decayed, missing, or filled surfaces (DMF)	1,137	1,259	632	163	1,769	1,422
New DMF surfaces per 100 surfaces at risk *	60.4	66.9	55.9	71.8	58.7	67.4
Number of missing teeth	74	84	45	19	119	103
Missing teeth per 100 teeth at risk *	18.0	20.3	18.8	32.8	18.3	21.9

* The differences between the cities are not statistically significant.

The mean caries-score for approximal surfaces of the matched groups also showed a difference between the Grand Rapids and Muskegon incidence of caries between 1944–1945 and 1956. This significant difference (0.05 level) was evidenced by clinical examinations alone, by X-ray alone, and by the combined technic (table 4). It is also apparent that the magnitude of the difference is essentially the same whether one uses the combined technic (clinical plus X-ray) or X-rays alone. For comparison, table 4 also includes the mean X-ray caries-scores for the total study group and for the unmatched children.

The occlusal and buccal+lingual surfaces in the matched study groups also had significantly lower caries-scores in Grand Rapids (table 5), so that these data also support the belief that fluoride confers protection on teeth erupted prior to the initiation of fluoridation. It is interesting to note that the occlusal surfaces in Grand Rapids had lower caries-scores (table 5) even though the number of surfaces affected was the same in the two cities (table 3). This supports the previous suggestion that the protection is obscured by the restoration of noncarious surfaces. Another interesting finding is the similarity of the magnitude of the decreases in mean caries-scores for the approximal (0.28) and the buccal+lingual surfaces (0.27). As the caries susceptibilities of these two types of surfaces are very different, this finding is curious but its significance is not immediately apparent.

SIGNIFICANCE

The findings that there were fewer and smaller cavities in Grand Rapids indicate that fluoride will have a caries-inhibitory effect on first permanent molars that have already erupted when fluoridation is initiated and support the more general hypothesis concerning the posteruptive effects of fluoride (*16*). However, the data probably underestimate the posteruptive caries-inhibitory effect on the whole dentition for two reasons. First, the Muskegon children did use fluoridated water for the last 5 years of the study period and it is probable that there was some inhibition of the caries process, particularly on the approximal surfaces of the first permanent molars in Muskegon. Second, other investigators (*27*, *28*) have shown that other tooth types are proportionately better protected than the molars. It is, then, reasonable to assume that incisors, canines, and premolars would show greater proportional changes in caries experience than were found in this study.

SUMMARY AND CONCLUSIONS

Clinical and bite-wing X-ray examinations were performed in April 1956, on the Grand Rapids and Muskegon public school children who in 1944–1945 were continuous residents and had two or more first permanent molars in eruption. Fluoridated water had been ingested by 184 of the Grand Rapids children since January 1945, and by 133 of the Muskegon children since July 1951. These children had satisfactory fluoride histories. The size of the cavities and restorations in first permanent molars were recorded by the clinical examiner at the time of examination and by the X-ray examiner after the X-rays from the two cities were randomized. A caries scoring system was used to put the data into a form that could be statistically tested and the comparability of the clinical and X-ray findings was investigated. The final analysis was based on new caries in teeth erupted prior to fluoridation in matched groups of 116 children each. These matched groups, which were balanced for caries susceptibility in 1944–1945, were selected from the children with satisfactory fluoride histories.

The data indicated that the Grand Rapids children consistently had fewer and smaller cavities than the Muskegon children. For all surfaces the mean caries-score was 17 percent lower in Grand Rapids, for approximal surfaces 14 percent lower, for buccal+lingual surfaces 31 percent, and for occlusal surfaces 11 percent lower than in Muskegon. The findings support the hypothesis that artificial fluoridation will inhibit caries in teeth that have erupted prior to fluoridation.

TABLE 3.—*Comparison of surfaces becoming decayed or filled between 1944–1945 and 1956 in first permanent molars erupted in 1944–1945, Grand Rapids and Muskegon matched groups, clinical examinations plus X-ray lesions of dentin*

	Occlusal Surface		Buccal+Lingual Surfaces		Mesial+Distal Surfaces	
	Grand Rapids	Muskegon	Grand Rapids	Muskegon	Grand Rapids	Muskegon
Number of new decayed or filled surfaces	226	226	194	239	402	437
Mean number of new decayed or filled surfaces *	1.95	1.95	1.67	2.06	3.46	3.77

* The differences between the cities are not statistically significant.

TABLE 4.—*Mean caries-scores for approximal surfaces becoming decayed or filled between 1944–1945 and 1956; first permanent molars erupted in 1944–1945, Grand Rapids and Muskegon*

	X-Rays Only			Clinical : Clinical+X-Ray	
	Total Group	Unmatched	Matched Group	Matched Study Group	
Muskegon	1.99	1.97	1.99	1.22	2.02
Grand Rapids	1.67	1.57	1.73	0.83	1.74
Difference	0.32	0.40	0.26	0.39	0.28
Standard error of difference	..	..	0.12	0.11	0.12

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Table 5.—*Comparison of mean caries-scores for surfaces becoming decayed or filled between 1944–1945 and 1956, first permanent molars erupted in 1944–1945, matched groups of Grand Rapids and Muskegon children, clinical+X-ray*

	Occlusal	Buccal+Lingual	Mesial+Distal	All Surfaces
Muskegon	2.08 ± 0.07 *	0.88 ± 0.08	2.02 ± 0.09	1.56 ± 0.07
Grand Rapids	1.85 ± 0.07	0.61 ± 0.07	1.74 ± 0.08	1.29 ± 0.06
Difference	0.23 ± 0.10	0.27 ± 0.11	0.28 ± 0.12	0.27 ± 0.09

* Standard error.

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A DENTAL CARIES STUDY. Comparing the Dental Health of DeKalb County Children After 5 Years of Fluoridation with Atlanta Children Who Had no Fluoride.

John E. Chrietzberg, Fred D. Lewis, Jr., James B. Carroll,
and T. Oscar Vinson

For the past five years continuous residents of DeKalb County have been drinking water containing small amounts of fluorides, while the continuous residents of Atlanta have drunk water containing practically no fluorides. Data in this report show that dental decay in DeKalb County children is definitely lower than in Atlanta children. This difference exists even though in DeKalb County the fluoride content was not maintained at the recommended level at all times, especially during the early stages of the operation. This inadequate fluoride concentration can perhaps be attributed to over-cautiousness on the part of the operators and water system officials, who are continuously hounded by the opposition to discontinue the process. These objectionists are suffering under the delusion that fluoridation is hazardous to the general health of the public.

Children examined in both Atlanta and DeKalb County have similiar socioeconomic backgrounds. The caries data for both groups were gathered under similar conditions by seniors of Emory University School of Dentistry and by U. S. Public Health Service Dental Interns. The large number of children in each age group adds soundness to the findings.

The benefits of fluoridation, as shown by the lower decay rates for permanent teeth of DeKalb County children, are greatest in the young group of 6–8 years of age as shown in table 1. The middle group of 8–10 years of age showed a slight benefit, while the older age group of 12–14 has had insufficient time to show a marked change. These findings bear out previous statements made by other investigators that the greatest benefit for the first five years of fluoridation could be expected among children of the younger age group whose teeth were in the formative stage when the procedure started.

CHART I

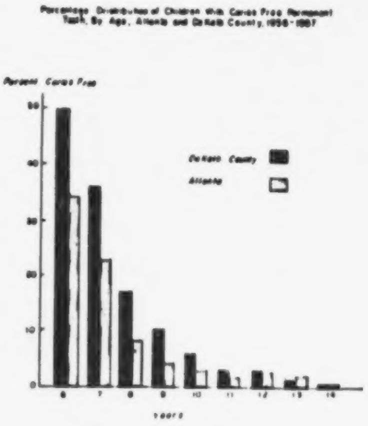

Chart I shows far more children with no decayed teeth in DeKalb County than in Atlanta. One half of the 6 year olds in DeKalb County have had no decay in their permanent teeth, while only one third of the Atlanta children of the same ages have had no decay. The 7 through 11-year old children show a similar pattern even though the tooth attack rate continues to climb with age for both Atlanta and DeKalb. The number of children of all ages with no decay experience will continue to increase and will eventually affect all groups as the fluoride project grows older in DeKalb County. On the other hand, the chances are that the Atlanta decay rates will remain static or perhaps increase if fluoridation is not started.

CHART II

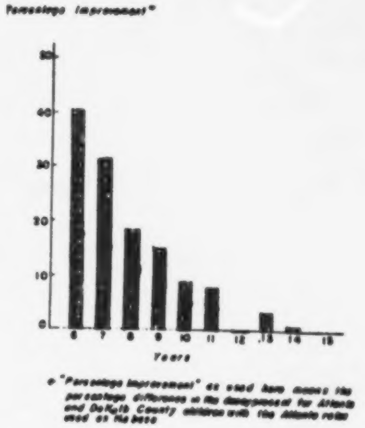

Chart II, using decay rates of the Atlanta children by age as a base, shows that the 6-year-old boys and girls of DeKalb County are 40 percent better off than the same age children in Atlanta. They are 31 percent better in the 7-year-olds and almost 20 percent in the 9-year-olds. Although the older age group shows little improvement over the Atlanta children, 10 years more of fluoridation can be expected to show a greater difference for all ages, which is in keeping with results of other studies.

The table and charts graphically illustrate the benefits observed after five years of fluoridation in DeKalb County, as compard to Atlanta with no fluorides. From these data the following conclusions can be drawn:

1. DeKalb County children in the 6–8-year age group have better teeth as a result of fluoridation than children in Atlanta who have been deprived of this health protection.
2. Children in the 9–11-year age groups,

TABLE 1.—*Showing number of children; average decayed, missing and filled teeth per child; percent reduction for the three groups of children who have been continuous residents of DeKalb County with fluoridated water and Atlanta area without fluorides—February 28, 1957*

Age by years	Number of children		DMF rates per child		% Improvement DeKalb
	Atlanta	DeKalb	Atlanta	DeKalb	
6–8	998	3859	2.25	1.64	27.1%
9–11	889	2884	4.23	3.72	12.1%
12–14	801	1530	8.41	8.00	4.9%
6–14	2688	8273	4.74	3.54	25.3%

whose permanent teeth were partially developed before fluoridation, show some improvement while the 12–14-year age group shows little or no improvement.

3. Atlanta has a higher percentage of children with one or more decayed teeth than DeKalb County children after 5 years of fluoridation.

4. As fluoridation continues, the older age children will show benefits similar to those in other studies, if the fluoride content is maintained in the DeKalb County water system at the recommended levels. These benefits will extend into the adult population when enough time has elapsed.

This preliminary report should serve as the forerunner of the more detailed report of the five year DeKalb County fluoridation project which will appear later in the *Journal of the Georgia Dental Association.*

This study was made possible through the cooperation of the Emory University School of Dentistry, the Dental Public Health staff of the USPHS Region IV Office and the public school officials of Fulton, Atlanta and DeKalb County. Acknowledgment of appreciation goes to Dr. William Kroschel and his USPHS staff who furnished the personnel for making the examinations in Atlanta and for their guidance in the planning and operation of the project and to Dr. John E. Buhler and his senior students who helped so efficiently in the DeKalb County examinations.

DENTAL CARIES IN MARYLAND CHILDREN AFTER 5 YEARS OF FLUORIDATION

A. L. Russell and Carl L. White

Marked inhibition of dental caries after 5 years of fluoridation has been demonstrated in a series of independent studies of school children (*1–8*). This report describes a similar finding in somewhat more minute detail.

The children under study are residents of Prince Georges and Montgomery Counties, Md., immediately adjacent to the District of Columbia. All are white; all were born and have resided continuously in the area, and none has received such caries-preventive treatment as the topical application of fluoride solutions. Characteristics of the population, methods employed, and some of the techniques of analysis have been described in detail in previous publications (*9, 10*). In brief, teeth were examined under adequate light with mirror and explorer by a small and carfully calibrated group of examiners. "Catches" were not counted as carious lesions in the absence of other indications of caries. Examinations have been made yearly since 1952, when the community water was fluoridated. The fifth year results are presented in this paper.

In 1952 the Washington Suburban Sanitary Commission maintained two filtration plants, which supplied the water used by the children in the study. The average daily fluoride content of finished water at one plant was 0.98 p.p.m. during the first year and 0.94 p.p.m. during the second; fluoride content of finished water at the other plant was 0.90 p.p.m. during both years. From January 1954 to January 1957, the average daily determination was 1 p.p.m. fluoride at both plants, with most monthly averages within the range of 0.97 to 1.03 p.p.m. fluoride. Since July 1955, all water has been processed in a single plant. The fluoridating vehicle has been sodium fluosilicate.

Mean numbers of teeth in eruption were virtually the same in 1957 as in

Table 1.—*Proportions of caries-free children at the outset of fluoridation and 5 years later, Montgomery and Prince Georges Counties, Md.*[1]

Age last birthday (years)	Numbers examined		Percent caries-free in deciduous dentition		Percent caries-free in permanent dentition	
	1952 (N=1,950)	1957 (N=2,872)	1952	1957	1952	1957
5	60	67	36.7	[2] 68.7	98.3	98.5
6	171	245	26.9	[2] 40.0	81.3	[2] 94.3
7	211	256	22.3	21.9	50.7	[2] 74.2
8	181	251	14.9	16.7	32.6	[2] 53.3
9	223	239	18.4	20.1	19.7	[2] 37.7
10	199	220	33.7	30.9	16.6	[2] 25.5
11	191	157	52.4	54.7	12.6	14.0
12	228	397	77.2	77.3	8.3	7.3
13	233	468	91.0	91.9	6.9	5.3
14	188	446	94.1	93.3	3.7	4.3
15	65	126	100.0	93.7	7.7	3.4

[1] All children are native born and are continuous residents of the area; none has received topical fluoride or other caries-preventive treatment.

[2] Difference significant at the $P=.01$ level.

1952. There were 1.2 percent fewer deciduous teeth present and 0.01 percent fewer permanent teeth present in 1957 than would have been expected on the basis of 1952 eruption data.

TOTAL CARIES EXPERIENCE

Differences in total caries experience in the children between 1952 and 1957 are shown in tables 1, 2, and 3 and figures 1 and 2.

Significantly more children were free of caries in deciduous teeth at ages 5 and 6, and significantly more were free of caries in permanent teeth at all ages from 6 through 10, in 1957 than in 1952 (table 1). At 5 and 6 years of age, 87 percent and 49 percent, respectively, more children had caries-free deciduous teeth. Differences in permanent teeth ranged upward from 16 percent at the age of 6, with the greatest difference in 9-year-old children. At this age, nearly twice as many children with caries-free permanent teeth were observed in 1957 as in 1952.

Mean numbers of deciduous and permanent teeth with caries experience (the def and DMF means, respectively) are shown in table 2 for each age group in each of the study years. In 1957, fewer def teeth per child were found in children up through the age of 7 years and fewer DMF teeth per child in all age groups, than in 1952. Differences were significant in deciduous teeth for children 5 and 6 years of age and in permanent teeth for children aged 6–11 years. Data for the 2 years are compared in figure 1.

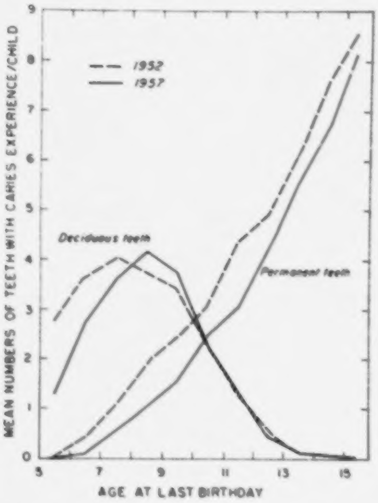

Figure 1.—*Mean numbers of deciduous and permanent teeth with caries in native-born children of Montgomery and Prince Georges Counties, Md., 1952 and 1957.*

The incidence of new carious permanent teeth for each age-cohort over the period of observation is pictured in figure 2. Children who were 10 years old in 1952, for example, had an average of 3.09 DMF teeth; at the age of 15 years, 5 years later, the average had risen to 8.14, a mean increment of 1.01 new DMF teeth per child per year. For children 9 years of age and younger, the incidence is shown from a zero point at the age of 5 years, the time at which permanent teeth begin to erupt. If a straight edge is laid along each of these incidence slopes in succession, it will be noted that the rate of caries increase is successively lower in each younger age group (see also table 3).

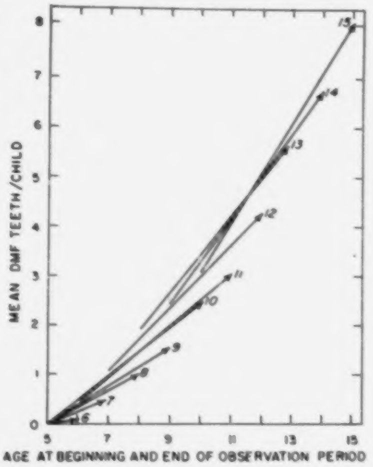

Figure 2.—*Increase in mean numbers of decayed, missing, or filled teeth in native-born children of Montgomery and Prince Georges Counties, Md., by age groups.*

Table 2.—*Mean numbers of def[1] deciduous and DMF[2] permanent teeth at the outset of fluoridation and 5 years later, Montgomery and Prince Georges Counties, Md.[3]*

Age last birthday (years)	Mean numbers of def deciduous teeth		Mean numbers of DMF permanent teeth	
	1952	1957	1952	1957
5	2.77	[4] 1.30	0.03	0.00
6	3.67	[4] 2.76	.41	[4] .10
7	4.04	3.68	1.09	[4] .54
8	3.78	4.19	1.90	[4] 1.05
9	3.43	3.74	2.42	[4] 1.59
10	2.30	2.31	3.09	[4] 2.49
11	1.29	1.36	4.39	[4] 3.04
12	.47	.48	4.96	4.28
13	.16	.14	6.15	5.64
14	.07	.07	7.66	6.73
15	.00	.06	8.57	8.14

[1] Decayed, extraction indicated, or filled.

[2] Decayed, missing, or filled.

[3] All children are native born and are continuous residents of the area; none has received topical fluoride or other caries-preventive treatment.

[4] Difference significant at the $P = .01$ level.

Table 3.—*Expected and observed incidence of new DMF[1] teeth in children using fluoridated water between 1952 and 1957, Montgomery and Prince Georges Counties, Md.*

Age interval (years)	Age at time of fluoridation	Incidence of new DMF teeth per child per year	
		Expected[2]	Observed
10–15	10	1.10	1.01
9–14	9	1.05	.86
8–13	8	.85	.75
7–12	7	.77	.64
6–11	6	.80	.53
5–10	5	.62	.50
5–9	4	.60	.40
5–8	3	.63	.35
5–7	2	.55	.27
5–6	1	.41	.10

[1] Decayed, missing, or filled.

[2] If 1952 rates had remained unchanged.

Mortality rates for permanent teeth were importantly lower in 1957 than in 1952, showing an overall decline at ages 6–13 of about 45 percent. But this change cannot be ascribed wholly to fluoridation, since a school dental health program was begun in the larger county shortly after the study began, and children examined in 1957 had, on the average, about one-third more filled teeth than were seen in children of the same ages in 1952. Filled teeth comprised 44.8 percent of the total number of DMF teeth in 1952 and 66.5 percent of the total number of DMF teeth in 1957.

PERMANENT FIRST MOLARS

In the 2 years under consideration, most of the DMF teeth observed in children through 10 years of age were permanent first molars. Considerably fewer DMF first molars were found in 1957 than in 1952, with the differences generally related to the age of the children at the time of fluoridation. Table 4 shows the numbers of DMF permanent first molars which would have been counted in 1957 had the 1952 attack rates remained unchanged; these numbers are compared with the actual numbers seen in children aged 5–12 years.

In table 5, caries attack rates for permanent first molars computed from the 1952 data are compared with attack rates actually observed during this fifth year of fluoridation in those children aged 6–12 years who were examined in 1956 and reexamined in 1957. Though more caries-free first molars were present in children of each age group in 1957, these

Table 4.—*Expected and observed numbers of carious first permanent molars in native-born children of Montgomery and Prince Georges Counties, Md., 1957*

Age group (years)	Age at time of fluoridation	Number of children (N=1,832)	Carious first molars expected [1] (Σ=3,529)	Carious first molars observed (Σ=2,820)	Difference (percent)
5	<1	67	2	0	−100. 0
6	1+	245	100	25	[2] −75. 0
7	2+	256	277	138	[2] −50. 2
8	3+	251	471	263	[2] −44. 2
9	4+	239	523	368	[2] −29. 6
10	5+	220	572	413	[2] −27. 8
11	6+	157	449	404	−10. 0
12	7+	397	1, 135	1, 209	+6. 5

[1] If 1952 caries attack rates had remained unchanged.
[2] Difference significant at the $P=.01$ level.

teeth were decaying at approximately the 1952 rates in children who were 4 years of age or older at the time of fluoridation. In children 3 years of age or younger at the time of fluoridation, attack rates were sharply and significantly lower than those seen in 1952.

In general, attack rates in mandibular first molars were more severe than attack rates in maxillary first molars, but both tended to follow the same patterns with age.

Older Children

Children 12 years old or older have been slower to exhibit a caries-inhibitory effect following fluoridation than younger children, when comparisons are based on lifetime caries experience. The incidence of new DMF teeth in children aged 13 or 14 years at the end of the third year of fluoridation in these Maryland counties has been reported in an earlier paper (*10*). At the time of fluoridation those children were 10 or 11 years old. No decrease in the incidence of caries was evident in that group during the 1954–55 year.

Similar data for the 653 children examined in 1956 and reexamined in 1957 who were 13 or 14 years of age at the end of the fifth year of fluoridation are shown in table 6. The differences between expected and actual numbers of teeth in eruption, in this group, were negligible; means of 26.33 and 27.38 teeth would have been expected on the basis of 1952 eruption data, and means of 26.24 and 27.35 teeth were actually observed. In 1957 there were slightly but significantly more DMF permanent first molars than had been seen in 1952. Means for the relatively caries-immune mandibular incisors and cuspids were essentially the same. Means for all other tooth types were significantly lower.

Crude incidence data may be computed from table 6. However, because of the greater numbers of teeth at risk, comparison with 1952 findings is more valid when based on attack rates (table 7). In general, the differences between expected attack rates and those observed during this fifth year of fluoride exposure are related to the age of the tooth at the time of fluoridation. Permanent first molars, for example, had been in eruption about 3 years when first exposed to

Table 5.—*Caries attack rates in first permanent molars, 1956–57, the fifth year of fluoridation, native-born children of Montgomery and Prince Georges Counties, Md.*

Age last birthday (years)	Expected [1]		Observed in children examined in 1956 and again in 1957			
	Mean teeth at risk [2]	Attack rate [3]	Number of children	Mean teeth at risk [2]	Attack rate [1]	Age at time of fluoridation
6	2. 733	13. 8	37	2. 756	[4] 2. 9	1+
7	3. 335	20. 1	192	3. 945	[4] 8. 4	2+
8	2. 897	27. 5	180	3. 236	[4] 13. 0	3+
9	2. 121	14. 6	186	3. 022	18. 0	4+
10	1. 812	22. 6	176	2. 171	24. 1	5+
11	1. 402	18. 6	116	1. 922	22. 9	6+
12	1. 089	0. 1	121	1. 248	30. 4	7+

[1] Computed from 1952 data.
[2] Number of teeth per child that were caries-free at the beginning of the year and those that erupted during the year.
[3] Percentage of teeth becoming carious during the year.
[4] Difference significant at the $P=.01$ level.

TABLE 6.—*Mean numbers of DMF*[1] *permanent teeth in 635 boys and girls, 13 and 14 years of age at the beginning and end of the fifth year of fluoridation (1956–57), Montgomery and Prince Georges Counties, Md.*

Type of tooth	Mean numbers of DMF teeth			
	Start of year		End of year	
	Expected[2]	Observed	Expected[2]	Observed
Maxillary				
Central incisor	0. 43	[3] 0. 30 ± 0. 025	0. 49	[3] 0. 39 ± 0. 028
Lateral incisor	. 43	[3] . 33 ± . 025	. 51	[3] . 40 ± . 027
Cuspid	. 04	[3] . 01 ± . 005	. 07	[3] . 02 ± . 006
First bicuspid	. 14	. 14 ± . 016	. 26	[3] . 19 ± . 019
Second bicuspid	. 14	. 11 ± . 014	. 24	[3] . 19 ± . 019
First molar	1. 36	[3] 1. 50 ± . 030	1. 50	[3] 1. 66 ± . 027
Second molar	. 40	[3] . 22 ± . 022	. 70	[3] . 43 ± . 028
All maxillary teeth	2. 93	2. 61	3. 78	3. 29
Mandibular				
Central incisor	0. 07	0. 08 ± 0. 015	0. 07	0. 11 ± 0. 017
Lateral incisor	. 07	. 06 ± . 012	. 08	. 08 ± . 014
Cuspid	. 01	. 01 ± . 005	. 02	. 02 ± . 007
First bicuspid	. 04	. 03 ± . 008	. 09	[3] . 05 ± . 010
Second bicuspid	. 14	. 11 ± . 016	. 23	[3] . 17 ± . 018
First molar	1. 62	1. 64 ± . 028	1. 67	[3] 1. 73 ± . 024
Second molar	. 73	[3] . 43 ± . 029	1. 07	[3] . 73 ± . 034
All mandibular teeth	2. 68	2. 36	3. 22	2. 87
All teeth	5. 61	[3] 4. 97 ± . 129	7. 00	[3] 6. 16 ± . 147

[1] Decayed, missing, or filled.

[2] If rates observed in 1952 had remained unchanged.

[3] Difference significant at the $P = .01$ level.

fluoride; the few remaining caries-free first molars were actually becoming carious at a faster rate than would have been predicted from 1952 findings. At the other end of the scale, permanent second molars, which erupted 2 to 3 years after fluoridation began, included significantly fewer carious teeth at the beginning of the year (see table 6), and attack rates during the year were sharply and significantly lower than expected. Attack rates were lower in all teeth erupting after fluoridation, but in only one of the teeth (the maxillary lateral incisor, with the shortest time at risk at that point) which were in eruption before fluoridation began.

Differences between expected and observed caries attack rates (omitting the mandibular incisors, teeth with attack rates too low for validity in a sample of this size) are shown in figure 3. In preparing this illustration teeth have been numbered from the midline toward the posterior part of the mouth. A bracket open at the top indicates position in the upper jaw, and a bracket open at the bottom indicates position in the lower jaw. The numeral 3 in a bracket open at the bottom, for example, indicates the mandibular cuspid.

DISCUSSION

So far as the deciduous dentition is concerned, little caries-inhibitory effect is evident in the data for children who were 2 years old or older at the time of fluoridation (7 years old or older in 1957). The most striking difference is shown in children aged 5 years in 1957, whose first waterborne fluoride exposure occurred during the period of calcification, and hence prior to eruption, of these teeth. Six-year-old children showed an intermediate degree of inhibition. Their deciduous teeth were fully calcified, but not in eruption, at the time fluoridation began.

Similarly, the younger the child at the time of fluoridation the greater the degree of caries inhibition in permanent first molars. In older children the greater degree of caries inhibition was seen in those tooth types first exposed to fluoride at the earlier stages in development. The critical point would seem to be the time of eruption. Tooth types with first fluoride exposure essentially coincident with eruption show minimal caries inhibition, and those in eruption 2 to 3 years at the time of fluoridation show no inhibitory effect which can be detected in counts of DMF teeth. In short, at this point (the fifth year of fluoridation) and measured in this way, the degree of caries inhibition in any tooth type seems to be a simple function of the length of fluoride exposure prior to eruption and the consequent risk of attack by dental caries.

Attack rates for children 13 or 14 years of age were slightly higher than expected during the third year of study (*10*), and they continued to be higher in permanent first molars in children of these ages in this fifth year. These facts suggest that (*a*) the prevalence of caries in this area may have been following an upward trend at the time the study was initiated, or (*b*) there may have been a gradual shift toward more stringent criteria over the course of the six yearly examinations.

Some such shift is inherent in the increased numbers of filled teeth. Bite-

TABLE 7.—*Expected and actual caries attack rates in 653 boys and girls 13 and 14 years of age during the fifth year of fluoridation, Prince Georges and Montgomery Counties, Md., 1956–57*

Type of tooth	Years at risk at start of fluoridation [1]	Mean DMF per child at start of year		Attack rate during year [3]		Total teeth becoming carious in year	
		Expected [2]	Observed	Expected [2]	Observed	Expected [2]	Observed
	Teeth in eruption and at risk of caries prior to fluoridation						
Mandibular central incisor	3. 08	0. 07	0. 08	0	1. 2	0	15
Mandibular first molar	2. 96	1. 62	1. 64	12. 0	[4] 24. 4	28	57
Maxillary first molar	2. 86	1. 36	1. 50	22. 5	[4] 31. 2	73	101
Maxillary central incisor	2. 03	. 43	. 30	3. 8	5. 3	42	59
Mandibular lateral incisor	2. 01	. 07	. 06	. 4	1. 1	5	14
Maxillary lateral incisor	. 95	. 43	. 33	5. 2	4. 2	55	45
Total	2. 32	3. 98	3. 92	3. 9	5. 5	203	291
	Teeth erupting after fluoridation						
Mandibular cuspid	−1. 06	0. 01	0. 01	0. 5	0. 2	7	3
Maxillary first bicuspid	−1. 27	. 14	. 14	6. 7	[4] 3. 1	81	37
Mandibular first bicuspid	−1. 29	. 04	. 03	2. 4	1. 0	31	13
Maxillary cuspid	−2. 02	. 04	. 01	1. 9	. 5	23	6
Maxillary second bicuspid	−2. 07	. 14	. 11	6. 0	4. 8	71	57
Mandibular second bicuspid	−2. 11	. 14	. 11	5. 1	3. 1	59	36
Mandibular second molar	−2. 57	. 73	. 43	29. 1	[4] 20. 3	280	196
Maxillary second molar	−3. 22	. 40	. 22	21. 5	[4] 13. 3	224	139
Total	−1. 95	1. 64	1. 06	8. 3	5. 2	776	487

[1] Measured from the median time of eruption to the mean age of the group at the time of fluoridation.

[2] If 1952 rates had remained unchanged.

[3] The percentage of teeth which were free of caries at the beginning of the year, or erupted during that time, which became carious during the year.

[4] Significantly different at the $P = .01$ level.

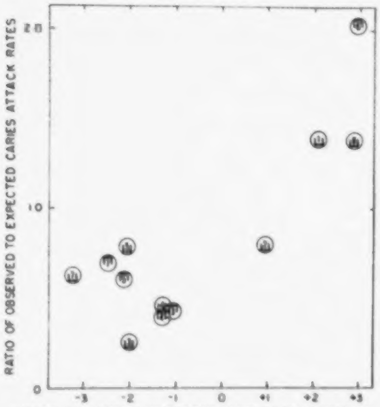

FIGURE 3.—*Differences between expected caries attack rates and rates observed during the fifth year of fluoridation, by eruption status of teeth, Montgomery and Prince Georges Counties, Md.*

wing X-ray examination is standard procedure in private dental offices in the area, which means that a proportion of these fillings represented lesions which could not have been detected by mirror and explorer until the following year.

In either event, the effect of such a shift would be toward a slight underestimation of the true magnitude of caries reduction.

SUMMARY AND CONCLUSION

A group of white, native-born children in Prince Georges and Montgomery Counties, Md., has been examined yearly since fluoridation of the community water supply in 1952. Their total caries experience after 5 years of fluoridation and the incidence of caries during the fifth year are compared with data from the baseline examination.

In this group at this time caries inhibition seems to be a simple function of the length of fluoride exposure prior to eruption of teeth and the risk of caries attack.

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DENTAL CARIES IN MARYLAND CHILDREN AFTER SEVEN YEARS OF FLUORIDATION

By A. L. Russell and Carl L. White

A substantial inhibition of dental caries after 5 years' use of fluoridated community water has been reported previously for children of Montgomery and Prince Georges Counties, Md., adjacent to the District of Columbia (*1*). At the end of the fifth year essentially all of the inhibitory effect was seen in teeth which had erupted after fluoridation was begun. The present report describes caries patterns in this same population 2 years later, after 7 years of fluoridation. Data presented here may be compared directly with the 5-year findings.

Design of the study and the methods employed have been described in detail (*2*). All the children in the study are white and were born in and have resided continuously in the area. None has received such caries-preventive treatments as the topical application of floride solutions. Examinations were made by a small and carefully calibrated group of examiners, using mirror and explorer. The survey described here was carried out in January and February 1959.

The area where these children live is supplied with water from a single filtration plant maintained by the Washington Suburban Sanitary Commission. The mean daily fluoride level in finished water at this plant was 1.005 ppm during 1957, the sixth year of the study, and 0.991 ppm during 1958. Most monthly averages were within the range of 0.99 to 1.02 ppm. Fluoride levels had been maintained at 0.9 to 1.0 ppm during the first 2 years of fluoridation and at 1.0 ppm during the third, fourth, and fifth years. The fluoridating vehicle used has been sodium fluosilicate (*1*).

As in 1957 the mean numbers of permanent teeth in eruption were essentially those which would have been predicted from the 1952 data. In the 2,872 children observed there were 0.2 percent fewer permanent teeth than in 1952. Numbers of deciduous teeth present were virtually identical with the 1952 expectancies in the 2,103 children 9 years of age or older. In children 8 years of age or younger, however, there were substantially more deciduous teeth present than would have been expected from the 1952 data, an increase over this age range of about one-half tooth per child. This was due principally to greater numbers of retained first and second deciduous molars. In children aged 6 years in 1959 (the first large group born since fluoridation to enter this study) there was an average per child of 0.13 deciduous first molars missing or indicated for extraction because of dental caries; the average for deciduous second molars was 0.17 per child. Similar statistics for 6-year-old children in 1952 are 0.30 first and 0.51 second deciduous molars. Neither of these teeth is shed normally during this age span; virtually all this loss is premature and is due to dental caries. Mortality rates for deciduous first and second molars were 57 and 67 percent lower in 1959 than in 1952.

TOTAL CARIES EXPERIENCE

Differences in total caries experience in the children between 1952 and 1959 are shown in tables 1, 2, and 3, and the chart.

Evidences of caries inhibition in deciduous teeth were confined almost wholly to children 8 years of age or younger in 1959 (table 1). These children had not reached their second birthdays at the time of fluoridation, and many of their deciduous teeth were not yet in eruption at that point. Through the age of 8 years there were about 63 percent more children free of caries in the deciduous dentition (273 compared with an expected 168), and mean numbers of deciduous teeth which were decayed, indicated for extraction because of dental caries, or filled (def) were importantly lower than those seen in 1952 (table 2). In children aged 9-15 years there were about the same proportions free of caries in the deciduous dentition and about the same mean numbers of def teeth as in 1952.

Benefits to the permanent dentition were evident in all age groups. There were significantly fewer permanent teeth which were decayed, missing, indicated for extraction because of caries, or filled (DMF) at all ages except 5 years, at which point few permanent teeth were in eruption. Numbers of children free of evidence of caries in the permanent dentition were significantly higher through the age of 11 years. The difference was of borderline significance ($P=.05$) at the age of 12. At the time of fluoridation permanent teeth were beginning to erupt in children aged 12 years in 1959; few or none were in eruption in younger children.

Fewer DMF teeth than would have been expected on the basis of the 1952 observations have occurred in each of the study groups during this 7-year span. Eight-year-old children in 1952, for example, averaged 1.90 DMF teeth per child; 15-year-old children averaged 8.57 per child. The apparent increment of DMF teeth over the 7-year span from age 8 to age 15 was 6.67 per child, a rate of 0.95 new DMF teeth per child per year. The actual increment from 1952 to 1959 in this age group was 4.76 new DMF teeth, an average of 0.68 new DMF teeth per child per year. Expected and observed increments for each of the age groups included in the 1959 survey are shown in table 3. In general, the younger the group at the time of fluoridation the greater the difference in incidence. The most striking differences are shown in

Mean numbers of deciduous and permanent teeth with caries in native-born children of Montgomery and Prince Georges Counties, Md., 1952, 1957, and 1959

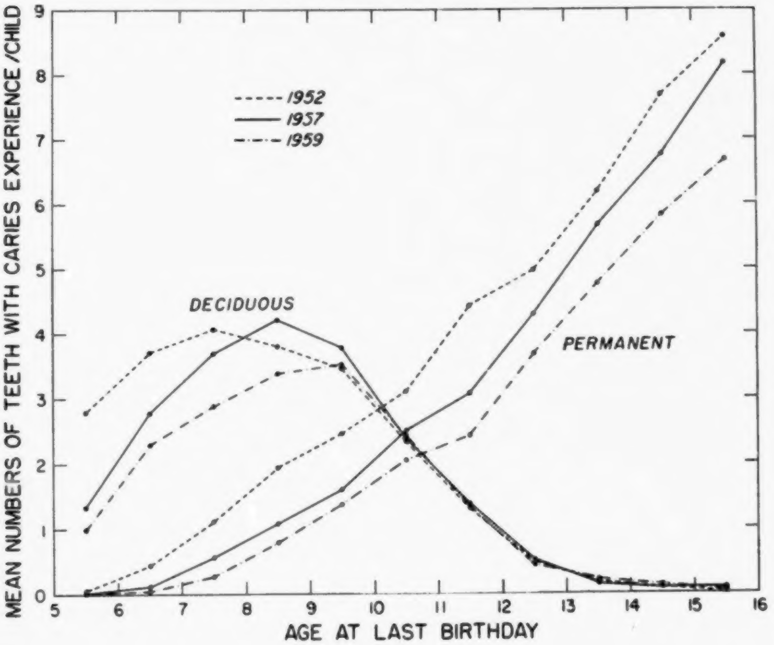

Table 1.—*Proportions of caries-free children at the outset of fluoridation and 7 years later, Montgomery and Prince Georges Counties, Md.*

Age at last birthday	Numbers examined		Percent caries-free in deciduous dentition		Percent caries-free in permanent dentition	
	1952 (N=1,950)	1959 (N=2,872)	1952	1959	1952	1959
5	60	31	36.7	[1] 67.8	98.3	100.0
6	171	244	26.9	[1] 43.4	81.3	[1] 94.7
7	211	257	22.3	31.5	50.7	[1] 85.6
8	181	237	14.9	[1] 27.4	32.6	[1] 61.2
9	223	223	18.4	19.3	19.7	[1] 43.5
10	199	226	33.7	31.4	16.6	[1] 29.2
11	191	218	52.4	50.5	12.6	[1] 24.3
12	228	451	77.2	76.5	8.3	13.5
13	233	382	91.0	89.5	6.9	7.9
14	188	451	94.1	94.0	3.7	4.7
15	65	152	100.0	95.4	7.7	3.3

[1] Significantly different from the 1952 finding at the $P=.01$ level.

children who were 2 years of age or younger at the time of fluoridation.

Permanent First Molars

Numbers of decayed, missing, and filled permanent first molars observed in children through 12 years of age in 1959 are shown in table 4. There were fewer DMF first molars in each age group than would have been predicted on the basis of the 1952 findings. Most of the benefit was seen in children aged 11 years or less; in this group of 1,436 children there were 1,060 fewer DMF first molars than would have been present in 1952. In general, the younger the age group at the time of fluoridation the greater the difference. Children aged 12 years in 1959, who received their first waterborne fluoride about the time these first molars were erupting, showed little reduction from the expected number.

Of these children—those aged 5-12 years in 1959—1,045 had also been examined in 1958. Their actual caries attack rates during the seventh year were likewise sharply lower than would have been predicted from 1952 data. In children aged 6 or 7 years, 5.0 percent of available first molars became carious for the first time during this period; in children aged 8 or 9 years, 11.4 percent; and in children aged 10 or 11 years, 13.8 percent. Attack rates for comparable age groups in 1952 were 17.0, 21.1, and 20.7 percent, respectively. Attack rates in children aged 12 years in 1959 were slightly higher than those computed for children of the same age in 1952.

Table 2.—*Mean numbers of def deciduous and DMF permanent teeth at the outset of fluoridation and 7 years later, Montgomery and Prince Georges Counties, Md.*

Age at last birthday	Mean numbers of def deciduous teeth		Mean numbers of DMF permanent teeth	
	1952	1959	1952	1959
5	2.77	[1] 0.97	0.05	0.06
6	3.67	[1] 2.35	.41	[1] .09
7	4.04	[1] 2.85	1.09	[1] .25
8	3.78	3.36	1.90	[1] .77
9	3.43	3.49	2.42	[1] 1.34
10	2.30	2.39	3.09	[1] 2.02
11	1.29	1.34	4.39	[1] 2.40
12	.47	.46	4.96	[1] 3.65
13	.16	.18	6.15	[1] 4.74
14	.07	.09	7.66	[1] 5.80
15	.00	.05	8.57	[1] 6.66

[1] Significantly different from the 1952 findings at the $P=.01$ level.

Table 3.—*Expected and observed incidence of new DMF teeth in children using fluoridated water between 1952 and 1959, Montgomery and Prince Georges Counties, Md.*

Age interval (years)	Age at time of fluoridation	Incidence of new DMF teeth per child per year	
		Expected [1]	Observed
8-15	8+	0.95	0.68
7-14	7+	.94	.67
6-13	6+	.82	.62
5-12	5+	.71	.52
5-11	4+	.73	.40
5-10	3+	.62	.40
5-9	2+	.61	.34
5-8	1+	.63	.26
5-7	<1	.55	.13
5-6	Unborn	.41	.09

[1] If attack rates observed in 1952 had remained unchanged.

Table 4.—*Expected and observed numbers of DMF first permanent molars in native-born children of Montgomery and Prince Georges Counties, Md., 1959*

Age group (years)	Age at time of fluoridation	Number of children (N=1,887)	DMF first molars expected (Σ=3,808)	DMF first molars observed (Σ=2,692)	Difference (percent)
5	Unborn.	31	1	0	−100.0
6	Unborn.	244	98	23	[1] −76.5
7	<1	257	282	65	[1] −77.0
8	1+	237	446	181	[1] −59.4
9	2+	223	489	295	[1] −40.0
10	3+	226	576	416	[1] −27.8
11	4+	218	624	476	[1] −23.7
12	5+	451	1,292	1,236	−4.3

[1] Difference significant at the $P=.01$ level.

Older Children

In this study special attention has been given children aged 12-14 years because this group has, in the past, been slower than younger children to exhibit a caries-inhibitory effect following fluoridation. During the third year of fluoridation no decrease in the incidence of new carious teeth could be demonstrated in children aged 13 or 14 years at the end of that year (*3*). There was a decrease in in-

Table 5.—*Mean numbers of DMF permanent teeth in 606 boys and girls 13 or 14 years of age at the end of the seventh year of fluoridation (1958–59), Montgomery and Prince Georges Counties, Md.*

Tooth type	Mean numbers of DMF teeth			
	Start of year		End of year	
	Expected [1]	Observed	Expected [1]	Observed
Maxillary				
Central incisor	0. 44	0. 19 ±. 021	0. 50	0. 28±. 026
Lateral incisor	. 45	. 21 ±. 022	. 52	. 28±. 026
Cuspid	. 04	. 003±. 002	. 07	. 02±. 006
First bicuspid	. 15	. 11 ±. 015	. 27	. 15±. 019
Second bicuspid	. 14	. 11 ±. 016	. 25	. 19±. 021
First molar	1. 37	1. 44 ±. 033	1. 51	1. 55±. 030
Second molar	. 42	. 21 ±. 022	. 71	. 38±. 028
All maxillary teeth	3. 60	2. 26	3. 83	2. 85
Mandibular				
Central incisor	0. 08	0. 05 ±. 012	0. 08	0. 08±. 015
Lateral incisor	. 07	. 04 ±. 013	. 08	. 06±. 013
Cuspid	. 02	. 000±. 000	. 03	. 01±. 005
First bicuspid	. 04	. 04 ±. 009	. 09	. 05±. 011
Second bicuspid	. 14	. 09 ±. 014	. 24	. 16±. 018
First molar	1. 63	1. 59 ±. 029	1. 67	1. 66±. 027
Second molar	. 76	. 36 ±. 028	1. 09	. 60±. 034
All mandibular teeth	2. 73	2. 16	3. 28	2. 62
All teeth	5. 72	4. 42 ±. 129	7. 11	5. 47±. 162

[1] If rates observed in 1952 had remained unchanged.

cidence during the fifth year in children of these ages. The decrease was confined essentially to the six tooth types which had erupted since fluoridation, amounting to a difference of about 12 percent in the total numbers of DMF teeth found at the end of that study year (*1*). At the end of the seventh year of fluoridation, this difference had increased to 23 percent, with a clear advantage accruing to each tooth type except the first molars and the mandibular incisors (table 5). Expectancies shown in this table differ somewhat from those computed for the fifth-year report, because the present study group differs from that seen 2 years ago in the ratio of boys to girls and in the distribution of ages.

Essentially the same numbers of teeth were in eruption in these older children as were seen in their counterparts in 1952. Had the 1952 rates persisted without change there would have been an average of 26.32 permanent teeth in eruption in these children at the beginning of the study year and 27.37 at the end. The actual means were 26.47 and 27.40 teeth, respectively.

Attack rates observed during the year in this group, together with rates which would have been observed had the 1952 patterns continued unchanged, are shown in table 6. As observed in the fifth year of fluoridation the degree of inhibition of caries is generally related to the developmental stage of the tooth at the onset of fluoridation. These children were 6 or 7 years of age when fluoridation was begun. Their first molars and their mandibular central incisors had been in eruption for an average of about 1 year at that time. Previous caries experience in these teeth and the numbers becoming carious during this seventh year were about what would have been predicted on the basis of the 1952 observations. The date of fluoridation coincided almost exactly with the median time of eruption of the maxillary central incisor and the mandibular lateral incisor. Only about half as many of these teeth as expected were carious at the beginning of the study year, but they were decaying at a somewhat faster rate than computed for children of the same ages in 1952, so that the margin of difference in these teeth can be expected to diminish with the passage of time.

Fluoridation preceded eruption by 1 to 5 years in all other tooth types. In these there were 48 percent fewer DMF teeth than expected at the beginning of the study year, and 36 percent fewer than

Table 6.—*Expected and actual caries attack rates in 606 boys and girls 13 or 14 years of age during the seventh year of fluoridation, Prince Georges and Montgomery Counties, Md., 1958–59*

Tooth type	Years at risk at start of fluoridation [1]	Mean DMF per child at start of year		Attack rate during year [3]		Total number of teeth becoming carious in year	
		Expected [2]	Observed	Expected [2]	Observed	Expected [2]	Observed
	Teeth in eruption and at risk of caries prior to fluoridation						
Mandibular central incisor	1. 10	0. 08	0. 05	0. 1	1. 4	0	16
Mandibular first molar	. 98	1. 63	1. 59	13. 1	17. 1	29	43
Maxillary first molar	. 88	1. 37	1. 44	22. 5	19. 6	85	67
Maxillary central incisor	. 05	. 44	. 19	3. 6	5. 3	32	58
Mandibular lateral incisor	. 03	. 07	. 04	. 5	1. 0	4	12
Total	. 61	3. 59	3. 31	4. 1	4. 8	150	196
	Teeth erupting after fluoridation						
Maxillary lateral incisor	−1. 03	0. 45	0. 21	5. 3	3. 9	46	42
Mandibular cuspid	−3. 04	. 02	. 00	. 6	0. 7	7	8
Maxillary first bicuspid	−3. 25	. 15	. 11	7. 0	2. 5	77	29
Mandibular first bicuspid	−3. 67	. 04	. 04	2. 5	0. 9	29	11
Maxillary cuspid	−4. 00	. 04	. 003	1. 7	0. 9	20	11
Maxillary second bicuspid	−4. 05	. 14	. 11	6. 3	4. 1	66	45
Mandibular second bicuspid	−4. 09	. 14	. 09	5. 7	4. 0	62	43
Mandibular second molar	−4. 55	. 76	. 36	28. 4	15. 7	201	148
Maxillary second molar	−5. 20	. 42	. 21	22. 0	10. 3	179	103
Total	−3. 61	2. 16	1. 13	7. 7	4. 5	687	440

[1] Measured from the median time of eruption to the mean age of the group at the time of fluoridation.

[2] If 1952 rates had remained unchanged.

[3] Percentage of teeth free of caries at the beginning of the year or erupting during the year which became carious during the year.

Table 7.—*Dental caries patterns in permanent first and second molars before fluoridation and those observed during the third, fifth, and seventh years of fluoridation, in children aged 13 or 14 years at the end of the study year*

Period	Mean number DMF at start of year	Mean number becoming DMF during year
	Permanent first molars	
Before fluoridation [1]	2. 98	0. 17
Third year	3. 07	. 23
Fifth year	3. 14	. 16
Seventh year	3. 03	. 18
	Permanent second molars	
Before fluoridation [1]	1. 13	0. 63
Third year	. 72	. 72
Fifth year	. 65	. 51
Seventh year	. 57	. 41

[1] Computed from 1952 baseline findings for an equal number of boys and girls at each age group, observed for exactly 1 year.

expected became carious during this term of observation.

All of the teeth reported for children aged 13-14 years during the fifth year (*1*) had been completely calcified prior to fluoridation. In this seventh-year group a few cuspids and second bicuspids and most second molars would still have been in the process of calcification at the time fluoridation began (*4*).

DISCUSSION

In these findings, as in those of previous years, there is no indication of a caries inhibition large enough, and persistent enough, to affect the count of decayed, missing, or filled teeth in tooth types which were already in eruption at the time fluoridation began. Caries patterns in the deciduous teeth of children aged 9 years or older were essentially the same in 1959 as in 1952. The general pattern is illustrated in table 7, which shows expectancies and observed caries findings in children 12 or 13 years old at the outset who were examined again 1 year later. In the 1952 data there were 2.98 first molars per child and 1.13 second molars per child decayed, missing, or filled at the outset. These means increased by an average of 0.17 first molar per child, and by 0.63 second molar per child during 1 year. First molars have continued to decay at about the same rates during this study; the pattern after 7 years of fluoridation is almost identical with that seen in 1952. It is obvious that neither fluoride nor any other caries inhibitor has effected any essential change in the caries susceptibility of first molars in these children over this 7-year span.

Susceptibility of second molars in these children, on the contrary, has systematically been diminished according to the elapsed time since fluoridation of the community water. Second molars observed during the third study year had erupted about the time of fluoridation While there were considerably fewer DMF second molars at the beginning of that year than had been seen in 1952, the attack rate observed during the year was somewhat higher than expected, so that the benefit gained may have been no more than a short delay in the time of onset of caries in these teeth. Children observed during the seventh year, by contrast, entered the year with only about half as many second molars decayed, and substantially fewer than expected became carious during the year. These teeth were in the final stages of calcification at the time of fluoridation. This pattern is consistent with the persistent and lasting inhibition of caries seen in adults who have lived in a natural fluoride area essentially all their lives (*5*).

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FIFTEENTH YEAR OF THE GRAND RAPIDS FLUORIDATION STUDY

By Francis A. Arnold, Jr., Robert C. Likins, A. L. Russell, and David B. Scott

The objectives of the Grand Rapids, Michigan, fluoridation study, and a series of interim findings have been described in previous reports (1-4). The fluoride ion content of the public water supply in Grand Rapids was adjusted to 1.0 part per million in January, 1945, and this level has been maintained within close limits ever since. Baseline dental caries data for virtually all Grand Rapids children between the ages of 5 and 16 were obtained during the 1944-45 school year. Dental examinations were made with mirror and explorer under artificial light, following standardized criteria which were designed to give evidence that penetration into the dentin had occurred before a lesion was classified as carious. Questionable surface defects, such as slight roughening of proximal surfaces or deep occlusal fissures were recorded, but these were not tabulated in reports as part of the totals for *DMF* (decayed, missing and filled) permanent teeth and *def* (decayed, extraction indicated and filled) deciduous teeth.

Of the more than 28,000 children examined 19,680 were born in Grand Rapids and had lived substantially all of their lives in the city. According to principles established earlier by Dean (5) the comparisons made throughout the study have dealt exclusively with continuous-resident white children. In 1945 similar baseline data were gathered for 4291 continuous-resident children of Muskegon, Michigan, with a similar but unfluoridated domestic water and for 5116 continuous-resident children of Aurora, Illinois, users of a water that for more than half a century contained an average concentration of 1.2 ppm of the fluoride ion supplied by nature. Following establishment of the baseline data, examinations of selected age groups were made annually over a 15 year period.

After six years of observation the caries prevalence in young Grand Rapids children had dropped significantly below that in the Muskegon control group, and had reached nearly the level that had been

Table 1.—*Distribution of continuous resident white children examined in Grand Rapids, Mich., and Aurora, Ill., according to age and year of examination.*

Age	Aurora, Ill. 1945	Grand Rapids, Mich.				
		Basic examination 1944-45	1956	1957	1958	1959
12	401	1,685	160	137	141	163
13	401	1,668	265	198	186	172
14	433	1,690	330	285	242	217
15	467	1,511	331	364	342	221
16	371	1,107	272	264	276	258
All ages	2,073	7,661	1,388	1,248	1,187	1,031

Table 2.—*Mean numbers of DMF permanent teeth per child, continuous resident white children examined in Grand Rapids, Mich., by age, year of examination, and elapsed time since fluoridation of the community water.*

Age	1944–45 Beginning of fluoridation	1956 After 12 years	1957 After 13 years	1958 After 14 years	1959 After 15 years	Per cent reduction in DMF teeth (1944–59)
12	8.07	2.91	3.36	3.36	3.47	57.0
13	9.73	4.47	3.86	4.66	3.58	63.2
14	10.94	6.02	5.48	5.55	5.38	50.8
15	12.48	7.52	6.79	6.59	6.22	50.2
16	13.50	8.87	8.13	8.30	7.03	47.9

Table 3.—*Dental caries prevalence in permanent teeth of continuous resident Grand Rapids children as observed in 1959 (15 years after fluoridation), compared with prevalance in children of Aurora, Ill., 1945–46.*

Age	Number examined	No. DMF teeth per child	Carious teeth per child*	Filled teeth per child*	Missing teeth per child
	Grand Rapids (in 1959, after 15 years of fluoridation)				
12	163	3.47	0.94	2.57	0.10
13	172	3.58	1.02	2.40	0.22
14	217	5.38	1.19	3.81	0.48
15	221	6.22	1.37	4.47	0.54
16	258	7.03	1.25	5.41	0.51
	Aurora, Ill., 1945, (1.2 ppm naturally occurring fluoride)				
12	401	2.95	2.06	0.96	0.12
13	401	3.09	2.10	1.20	0.14
14	433	3.64	2.05	1.58	0.18
15	467	4.54	2.37	2.05	0.35
16	371	5.19	2.32	2.71	0.41

* A tooth both filled and carious is counted in each column.

observed in Aurora, where fluoride occurred naturally in the water. These findings, taken together with evidence of similar trends in other fluoridation studies stimulated considerable local pressure for the institution of a fluoridation program in Muskegon at the earliest possible date. The city officials agreed and fluorides were introduced to this water supply in July, 1951. Examinations were continued for 3 more years in Muskegon, however, and caries prevalence as measured by DMF means was substantially lowered in children aged 8 years or younger at the end of this period (3). In subsequent analyses of Grand Rapids data comparisons have been made with the original Grand Rapids findings and with those for Aurora (4).

By 1954 (3) Grand Rapids children 9 years of age or younger who had been using the fluoridated water from birth, exhibited a decreased caries prevalence similar to that observed in Aurora children of the same ages. Very nearly the same degree of inhibition was seen in children 10 or 11 years of age, who were two years old or younger at the beginning of fluoridation (3). It was decided at this point that further annual sampling of groups through age 11 would be redundant. Thus, during the last period, 1955-59 (11th through 15th years of fluoridation), the study was confined to children of Junior High School and High School ages. Data for the 11th year have been presented previously (4). The present report is primarily a description of findings over the final period of observation. 1956 through 1959.

RESULTS AND DISCUSSION

The composition of the population sample is shown by age in Table 1 and the mean numbers of DMF permanent teeth per child for these groups are presented in Table 2. Children aged 14 years or younger in 1959 were born after fluoridation was begun; those 15 years of age were less than one year old at the time of fluoridation; and those 16 years of age in 1959 were in the second year of life when fluoridation was started. All showed substantially lower DMF means than were observed in Grand Rapids in 1944-45. Children aged 12, 13, or 14 years, with fluoride usage from birth, showed means which were 57%, 63%, and 51% lower, respectively. Children aged 15 and 16 years had means lower by about 50%.

Detailed findings for the fifteenth year are presented together with the 1945 data from Aurora in Table 3. The level ultimately reached in Grand Rapids was nearly as low as that observed at the outset in Aurora where the average fluoride level, 1.2 ppm F, was 20 per cent higher than the level maintained throughout the study in Grand Rapids. The relation between filled and carious teeth per child in Grand Rapids in 1959 and in Aurora in 1945 showed some interesting variations. There were nearly twice as many unfilled carious teeth in Aurora in 1945 as in Grand Rapids in 1959. Oppositely, there were more than twice as many filled teeth per child in Grand Rapids in 1959 as in Aurora in 1945.

An analysis of the cumulative data has indicated that progressively more dental treatment has been received in all groups examined. In 1944-45, roughly half of the DMF teeth per child in the 12-16 year age groups were recorded as filled in Grand Rapids, Muskegon, and Aurora. At the tenth study year, in 1954, when the caries prevalence rates had dropped appreciably in Grand Rapids, but not in Muskegon, nearly two thirds of the DMF teeth in children of the same ages were recorded as filled in both cities.

Comparison of the DMF rates alone suggests that, while there was a great reduction in caries prevalence in Grand Rapids over the fifteen year period, the level ultimately reached was not quite as low as that observed in Aurora at the beginning of the study. An examination of the figures for the individual DMF components, however, indicates that such an interpretation may not be entirely warranted. Mean numbers of missing teeth were nearly identical; corrected to the same age distribution as that of children in Aurora, the Grand Rapids mean for the whole group is 0.37 teeth per child, as against 0.36 in Aurora. The relationship between filled and carious teeth per child in Grand Rapids in 1959 and in Aurora in 1945, on the other hand, showed some interesting variations. There were nearly twice as many unfilled carious teeth in Aurora in 1945 as in Grand Rapids in 1959. Oppositely, there were more than twice as many filled teeth per child in Grand Rapids in 1959 as in Aurora in 1945, and since the numbers of teeth involved in this difference were of a greater magnitude, it is this component that accounts for the higher DMF rates in Grand Rapids. In explaining these differences in carious and filled tooth rates consideration must be given to advances in dental practice during the period of 14 years that elapsed between these two sets of examinations. An analysis of the cumulative data has indicated that progressively more dental treatment has been received in all groups examined. In 1944-45, roughly half of the DMF teeth per child in the 12-16 year age group were recorded as filled in Grand Rapids, Muskegon and Aurora. At the tenth study year, in 1954, when the

Table 4.—*Increments of new DMF teeth per child in cohorts of children examined in Grand Rapids in 1954 and in 1959, with five-year expectancies based on 1944-45 findings.*

Age in—		Mean DMF in—		Five-year increment per child	Mean increment per year	Expected mean annual increment*	Difference, per cent
1954	1959	1954	1959				
7	12	0.69	3.47	2.78	0.56	1.24	−54.8
8	13	1.27	3.58	2.31	0.46	1.36	−66.2
9	14	1.97	5.38	3.41	0.68	1.41	−51.8
10	15	2.34	6.22	3.88	0.78	1.51	−48.3
11	16	2.98	7.03	4.05	0.81	1.42	−43.0

*If caries attack rates observed in 1944–45 had persisted without change.

caries prevalence rates had dropped appreciably in Grand Rapids, but not in Muskegon, nearly two thirds of the DMF teeth in the same age groups in both cities were recorded as filled. In Grand Rapids, in 1959, the fifteenth study year, about three quarters of the DMF teeth were recorded as filled. The early detection of proximal lesions by means of X-ray examination was standard practice in 1959, but not necessarily so in 1944. Thus, some of the filled lesions included in the 1959 Grand Rapids DMF totals would have gone undetected or have been recorded as "preclinical" or "questionable" lesions in 1944, and would not have constituted a part of the DMF rates as reported. Conversely, had the Aurora examinations been done in 1959, the DMF rates might have been somewhat higher and the ratio between filled and carious teeth might have approximated more closely that observed in the same year in Grand Rapids.

Children aged 12 through 16 years in 1959 were cohorts of children (and in most cases the same) aged 7 through 11 years in 1954. An estimate of the increment of new DMF teeth for any of these age groups can be made by comparing the number of DMF teeth in 1959 with the number of DMF teeth in the cohort at the beginning of the five-year period. Seven-year-old children examined in 1954, for example, had an average of 0.69 DMF teeth per child. In 1959 these children had reached the age of 12 years and the DMF mean had risen to 3.47 per child. This was a total increase of 2.78 DMF teeth per child over this five-year span, for an average increase of 0.56 new DMF teeth per child per year. These computations, for each of the cohorts, are shown in Table 4. The whole group of children examined in 1959 would have experienced about 1.39 new DMF teeth per child per year between 1954 and 1959 if caries rates seen in Grand Rapids prior to fluoridation had continued without change. The actual increase was 0.66 new DMF teeth per child per year, a difference of 52.5 per cent. In children born after fluoridation (those aged 12-14 years in 1959) the expected mean increment was 1.33 new DMF teeth per year and the observed increment was 0.57 teeth, a difference of 57.1 per cent. In children younger than two years of age at the time of fluoridation the expected and observed increments were 1.47 and 0.79, respectively, a difference of 46.3 per cent.

No such dramatic and persistent inhibition of caries in large population groups had ever been demonstrated by any other means than fluoridation of a domestic water supply. While there were marked decreases in caries attack rates of European children during World War II, these were associated with restriction of sugar allowances to emergency levels (6), and gradually rose again to prewar levels after sugar rationing was ended (7). In an independent Maryland study covering the period from 1952 to 1959, it was found that permanent first molars which were in eruption at the time of fluoridation of the community water continued to decay at the same rate throughout the seven-year term of observation (8). The curves in Figure 1 demonstrate clearly that children aged 7-11 years not only entered the final five-year period of observation with many fewer carious teeth than their counterparts in 1944-45, but that caries attack rates continued to be sharply less than those observed at the outset of fluoridation.

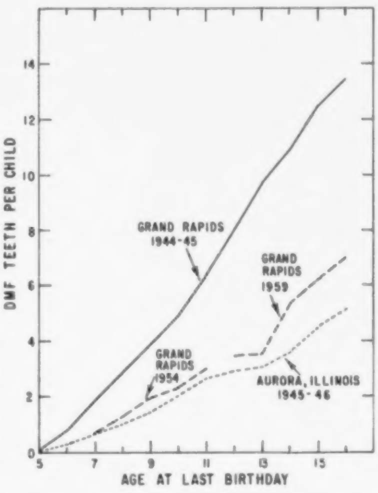

FIGURE 1.

The statistical validity of the reported difference and the possible effects of examiner variation on the conclusions that have been reached have been given special attention. Three dental officers participated in the 1959 examinations. The same qualitative and very nearly the same quantitative conclusions are supported by the findings of any one or any two of the three men that are presented here as findings for the whole team. Each set of figures for each examiner is statistically different from the 1944-45 means for each age group and by astronomical odds. In the smallest individual sample of only 32 twelve year olds, the odds are about 2,500,000 to one that the difference is not due to chance. In this large sample study, with the differences so great, it has been deemed redundant to list statistical probabilities. Standard errors for means in the data previously published, however, can be readily computed by Grainger's Q with negligible departures from the actual values (9).

Dental fluorosis, when observed, was recorded and classified according to Dean's criteria (5). These findings, shown in Table 5, are very similar to those reported for younger children after 11 years of fluoridation (4). Four of 1,031 continuous-resident children exhibited

Table 5.—*Amount and degree of dental fluorosis observed in Grand Rapids, Mich., after 15 years of fluoridation.*

Age	Number of children examined	Fluorosis classification							
		Normal		Questionable		Very mild		Mild	
		No.	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*
12......	163	143	87	12	7	8	6	0	0.0
13......	172	148	86	18	10	5	3	1	0.6
14......	217	191	88	16	7	7	3	3	1.4
15......	221	206	93	12	6	3	1	0	0.0
16......	258	234	91	19	7	5	2	0	0.0
All ages......	1,031	922	89.4	77	7.5	28	2.7	4	0.4

mild fluorosis; none of these cases was obvious enough to impair appearance. About 10 per cent of the total group showed the nearly-imperceptible signs associated with questionable or very mild fluorosis. About the same total percentage of enamel opacities of all types was observed in the 1,112 children not included in the study group because they had been born in another city or had lived in some other place than Grand Rapids for an extended period. About half of these opacities were classified as questionable or very mild fluorosis with three children (0.3 per cent) classified in the "mild" category.

SUMMARY

1. The dental caries status of continuous resident children of Grand Rapids, Michigan, aged 12 through 16 years, was determined for the twelfth through fifteenth years of fluoridation.

2. During the last period of observation caries attack rates were lowered by 57 per cent in children born after fluoridation (aged 12-14 years in 1959) and by 46 per cent in children less than two years of age at the time of fluoridation (aged 15-16 years).

3. After 15 years total caries experience was lowered by 50 to 63 per cent in children aged 12-14 years, and by 48 to 50 per cent in children aged 15 or 16 years.

4. No undesirable cosmetic effects due to dental fluorosis have been observed.

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Engineering Problems In Water Fluoridation

Fluoridation of drinking water requires the accurate maintenance of a constant and invariable concentration of fluoride in a community water supply. Among the chemicals used for this purpose are sodium fluoride, sodium silicofluoride, ammonium silicofluoride, hydrofluosilicic acid and fluorspar (calcium fluoride).

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FLUORIDATION OF PUBLIC WATER SUPPLIES

By F. J. Maier

Some of the most important contributions to the improvement of public health have been made through the application of procedures designed to remove from drinking waters substances injurious to health. Conversely, the discovery of the role of optimum amounts of fluorides in water has led to the concept that the treatment of drinking water might include the addition of specific substances to prevent disease.

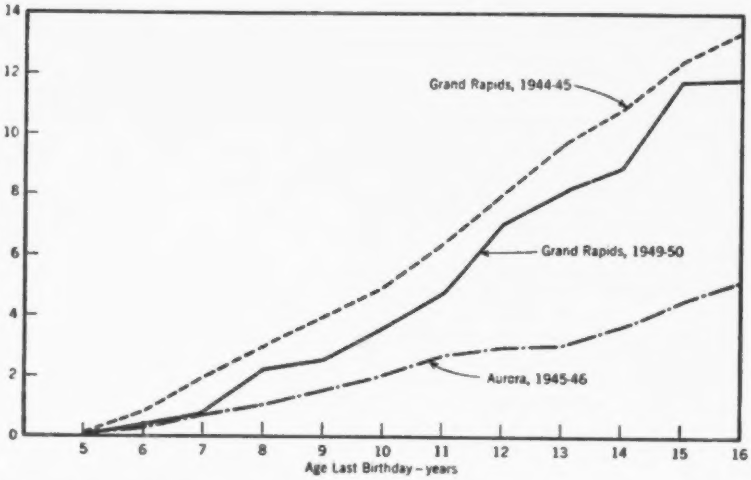
Fluorides are added to drinking water in order to reduce tooth decay. Where necessary, fluorides are removed from water to prevent dental fluorosis. That the removal of fluorides from water will prevent one disease, while their addition to water will prevent another entirely different defect from developing, is one of the most curious reversals in the history of water supply engineering.

The role of fluorides as the causative agent of dental fluorosis has been known since 1931, although a description of the lesion had been reported 30 years previously. Fluorosis in its mildest forms produces chalky-white spots or causes the entire crown surfaces to turn chalky white. As fluoride concentrations increase, secondary, brown to black stains appear, and, in the severest cases, the enamel shows a pitted and eroded surface which chips and crumbles easily, resulting in a degree of enamel attrition not commensurate with the chronological age of the individual affected.

Fluorosis becomes evident in children if, during the period of enamel calcification, they have been consuming water which contains more than about 1.5 p.p.m. of fluoride ion. The effects of fluoride concentrations in water will differ, of course, as the amounts of water consumed and the susceptibility of individuals vary. The lesion becomes more severe as the concentration of fluorides increases, until, at 6.0 p.p.m., practically all children are affected, many with a marked to severe type causing considerable disfigurement.

On the other hand, it has been shown that 1.0 p.p.m. fluoride naturally occurring in water is sufficient to decrease the incidence of dental caries by more than 60 percent. The accompanying degree of fluorosis is negligible.

Studies are now in progress to demonstrate that this reduction in caries incidence can be duplicated by artificially adding fluorides. The oldest of these studies, started in January 1945 at Grand Rapids, Mich., with 1.0 p.p.m. fluoride, has shown recently that the incidence of caries experience among children aged 5 to 7 is practically the same as that in children in the same age groups at Aurora, Ill. (fig. 1). The fluoride content of the Aurora water supply is 1.2 p.p.m. There are no discernible reasons for suspecting that each successive yearly examination at Grand Rapids will not follow the same trend. There is no evidence, either, that 1.0 p.p.m. of the fluoride ion naturally occurring in water is different in effect from the same concentration derived by dissolving a fluoride compound in water.



* Decayed, missing and filled.

Figure 1.—*Caries experience at Grand Rapids and Aurora.*

Similar caries reductions have been noted at other places where fluorides are being added. For example, at Newburgh, N.Y., where fluoridation was started in May 1945, a 30 percent reduction in caries in permanent teeth was found among children in the 6-to-12 age group.

At Sheboygan, Wis., where fluoride was first added in February 1946, a 20 percent reduction was shown in 3 years. At Brantford, Ont., a 22 percent reduction in decay rates of the permanent teeth was reported after 40 months of fluoridation. There are, in addition, more than 40 other places in the United States where fluoridation is practiced, most of them being in Wisconsin.

FLUORIDE CONCENTRATION

In most of the earliest fluoridation projects, a concentration of 1.0 p.p.m. or slightly higher has been used. This rate of feed is based essentially on the conclusions reached by H. T. Dean of the U.S. Public Health Service in his study of 7,257 selected children in 21 cities in Illinois, Indiana, Ohio and Colorado (*1, 2*). A fluoride concentration of 1.0 p.p.m. is probably the optimum for this north central area of the country, but not necessarily the best for all areas.

The severity of fluorosis and the degree of caries protection varies (within limits) with the fluoride intake. The quantities of fluoride ingested are generally dependent on the amounts of water consumed. Consumption, in turn, hinges in part on the temperature and relative humidity of the locality. It has been suggested that the optimum fluoride rate of feed is the highest concentration which will not produce more than about 10 percent fluorosis. A 10 percent incidence of endemic fluorosis represents the very mildest degree and, therefore, is of no esthetic consequence; it is, in fact, generally unrecognizable.

What is needed, then, in order to determine the optimum rate of feed for a given locality, are sufficient data from

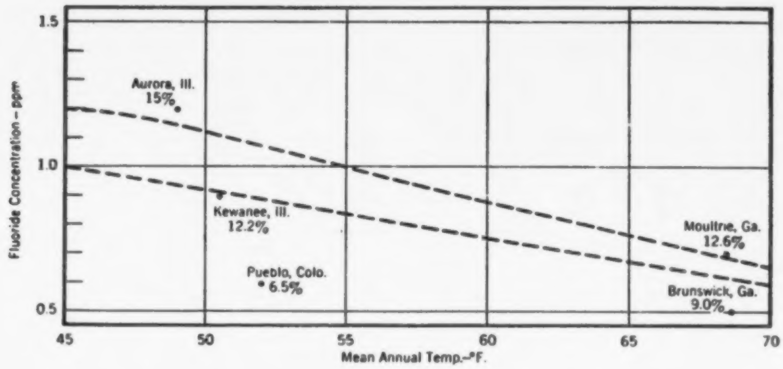


FIGURE 2.—*Temperature and optimum fluoride content.*

places where public water supplies contain 0.5–1.5 p.p.m. fluoride; where the fluorosis prevalence is about 10 percent among children at least 14 years of age; where reliable data on humidity and mean annual temperatures are available; and where there is a sufficient number of children to justify a valid conclusion. Unfortunately, such data have been obtained from too few such communities to formulate definite conclusions. Nevertheless, all available information has been plotted in figure 2 to indicate very roughly the possible relationship between the optimum fluoride content of municipal water supplies and mean annual temperatures (*1, 2*). Also shown in figure 2 is the percentage prevalence of fluorosis found at the various cities noted.

Perhaps the most that can be ascertained now from such a curve is that those places which experience a mean annual temperature of more than 55°–60° F. should supply water containing less than 1.0 p.p.m. fluoride. Mean annual temperatures alone may not, however, be the best basis for estimating water consumption in some communities. For example, more water may be consumed in an area where the days are very hot and the nights cold than in a locality with a more uniform temperature but the same mean annual temperature. Relative humidity is also an important factor to be weighed. Data on considerably more communities will have to be accumulated before this suggested curve can be regarded as entirely practicable. Eventually it will be possible to construct a graph from which, when the local mean annual temperature (or mean daytime temperature) and relative humidity are known, the optimum fluoride feed can be read directly.

At Charlotte, N.C., Z. M. Stadt, Dental Officer, City Health Dept., has concluded that an equivalent of 0.9 p.p.m. fluoride should be added. The mean annual temperature at Charlotte is 61.9° F. As shown in figure 3, Stadt and R. S. Phillips, superintendent, Charlotte Water Department have gone further and correlated average monthly temperatures, probable variations in water intake (based on carbonated-beverage sales) and the resulting variable fluoride feed (*3*).

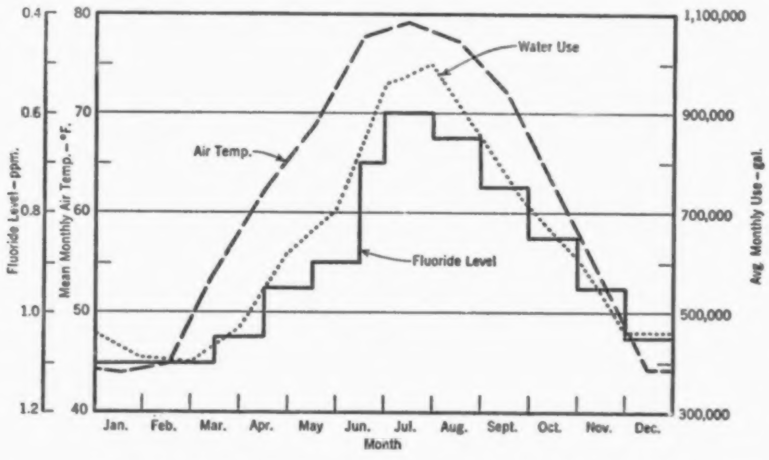


FIGURE 3.—*Water consumption, air temperature and fluoride level.*

A fluoride concentration which is set too high, although providing increased caries protection, will cause an excessive amount of fluorosis and, possibly, result in an embarrassing series of lawsuits. One such incident might well discourage fluoridation projects scheduled for many other communities.

FLUORIDE COMPOUNDS

Early fluoridation projects employed sodium fluoride as the source of fluoride. This compound was adopted because it was commercially available in large quantities, was convenient to use and had a solubility which was relatively high and constant over a wide temperature range; also, studies on its toxicity and physiological effects had been completed.

Obviously, there are many other compounds which might be used. Table 1 lists, in order of solubility, those fluoride compounds which, because of their present availability, cost, purity and freedom from ordinarily undesirable ions, appear to be suitable for fluoridation. There are, of course, other compounds (potassium fluoride, magnesium fluoride, sodium bifluoride) which may become more attractive in the future. Some fluorides have obvious advantages over others. For instance, the calcium salt is the cheapest source of fluoride, but because of its insolubility it would be difficult to feed. It might well become the preferred fluoride, however, in some of the larger plants, where it could be fed directly into the water.

The acid fluorides (particularly hydrofluoric acid), although considerably more expensive, can be fed directly without considering solubility. At Madison, Wis. (*4*), 70 percent hydrofluoric acid is used in order to conserve space in a series of small well pumphouses. The acid is proportioned indirectly with a solution feeder. Instead of pumping the acid (which is extremely corrosive), the feeder forces an inert mineral oil into the top of the acid tank. The oil, riding on the acid, displaces a volume of acid equal to the amount of oil pumped. Corrosion difficulties in piping and fittings appear to have been overcome by using Monel metal or hard rubber. Because of its corrosiveness and the danger inherent in its handling, hydrofluoric acid should not be used in

TABLE 1.—*Characteristics of fluoride compounds*

Chemical	Formula	Fluoride in Pure Chemical	Purity of Commercial Chemical	Fluoride in Commercial Chemical	Solubility at 25°C. *g./100 ml.*	Fluoride in Satd. Soln. at 25°C. *ppm.*	Amt. of Soln. per mil.gal. of Water (25°C., 1.0 ppm. F^-) *gal.*	Cost per Pound ¢	Cost per Pound of Commercially Available F^- ¢
		per cent							
Calcium fluoride	CaF_2	48.8	98.0	47.7	0.004	19.1	52,500	2.0	4.2
Sodium silicofluoride	Na_2SiF_6	60.64	98.8	60.0	0.762	4,572	219	3.23	5.4
Sodium fluoride (90 per cent)	NaF	45.3	94	42.5	4.05	17,200	58.2	10.5	24.8
Sodium fluoride (98 per cent)	NaF	45.3	98	44.4	4.05	18,000	55.6	11.5	26.0
Hydrofluosilicic acid	H_2SiF_6	79.0	30	23.7		Fed directly	3.35*	5.0	21.0
Hydrofluoric acid	HF	95.0	60	57.0		Fed directly	1.42†	13.5	23.7

* Specific gravity 1.26.
† Specific gravity 1.24.

TABLE 2.—*Solubility of Sodium Silicofluoride*

Temp. °F.	Solubility *oz./gal.*
32	0.58
50	0.72
77	1.02
95	1.25
104	1.40
113	1.50
122	1.62
131	1.78
140	1.98
176	2.60
212	3.26

water plants unless the most expert supervision is available.

Balancing cost against convenience in handling, it appears that sodium silicofluoride might be the most promising source of fluoride at present. Racine, Wis., has been able to purchase it at 3.23 cents per pound delivered, which, from the standpoint of cost per pound of available fluoride, makes it about one-fifth as expensive as the fluoride obtained from sodium fluoride.

Most of the sodium silicofluoride produced today is a by-product of the phosphate salt and phosphate fertilizer industries. It is derived from phosphate rock (containing calcium fluoride and fluoapatite) which has 3-4 percent fluorine. Silicon tetrafluoride, evolved when the rock is acidified, can be converted to hydrofluosilicic acid by reacting with water, but in most plants it is allowed to escape into the atmosphere or is absorbed in waste water. By reacting the hydrofluosilicic acid with a sodium salt, sodium silicofluoride is formed. It is estimated that over eight times the present annual consumption (10,000 tons) of sodium silicofluoride could be produced at the present rate of phosphate salt production (*5*). It appears then, that, from this source alone, there is enough fluoride now available to add 1.0 p.p.m. to the water consumed by the entire population of the United States. Recent, unpublished studies by F. J. McClure have shown that the availability of the fluoride ion in sodium fluoride is comparable to that in sodium silicofluoride.

Sodium silicofluoride is a crystalline, nonhygroscopic salt with a molecular weight of 188.05, a density of 2.679, a density of saturated solution of 1.0054 and a pH of saturated solution of 3.5–4.0. The hydrolysis permits direct analytical determination by titration with a standard alkaline solution. The solubility at various temperatures is shown in table 2. Two grades are available commercially, regular (72 lb. per cubic foot) and fluffy (55 lb. per cubic foot). Both sodium fluoride and sodium silicofluoride can be purchased in 100-lb. bags, 375-lb. fibre drums and 425-lb. barrels.

The relatively low and variable solubility of the salt will make it somewhat more difficult to feed as accurately as sodium fluoride. Solution feeders will require either a relatively large solution tank (219 gal. (at 25° C.) per million gallons of water treated) or a smaller tank with the salt fed as a slurry. Feeding a slurry requires constant agitation of the mixture and a design for the feeder valves and slurry lines which will prevent clogging. If hot water is continuously available (particularly at softening plants employing carbonation), a constant high-temperature water can be utilized, which will result in lower solution water requirements. For dry feeders, a larger solution box requiring more water will be necessary, as compared with a feeder using sodium fluoride.

FLUORIDE FEEDERS

Devices for feeding fluorides accurately have generally been adapted from those machines originally designed for feeding a variety of chemicals in water treatment and industrial plants. Feeders can be broadly divided into two types: (1) solution feeders, where a measured quantity of accurately prepared fluoride solution is delivered during a specified period; and (2) dry feeders, where a predetermined quantity of the solid material is delivered during a given time interval. The choice of a feeder depends on the rate at which fluoride is required. This rate, in turn, is determined by the water consumption and the fluoride content of the untreated water. Solution feeders are used for smaller supplies and dry feeders for larger ones. There is, of course, a wide area within which either type would be equally successful.

Solution feeders in single units have heretofore been used for supplies delivering up to about 2.0 mgd. Such units have a maximum delivery rate of about 400 ml. per minute. When sodium fluoride is used, the solution strength is usually brought up to 3.0–3.5 percent, although this concentration can be made much lower, if desired, where the water supply is very small. If excessive amounts of calcium or magnesium are present in the solution water, calcium fluoride or magnesium fluoride will precipitate, settle out and become lost. To prevent this in a saturated fluoride solution, the solution water should not contain more than 20 p.p.m. calcium (50 p.p.m. as $CaCO_3$) at 25° C. The concentration of magnesium may reach 50 p.p.m. (210 p.p.m. as $CaCO_3$) at the same temperature before precipitation occurs.

A more concentrated solution of sodium fluoride can be obtained with a recently developed saturator, in which water passes down through a bed of crystalline sodium fluoride (*6*). The sodium fluoride rests on a straining device so that a saturated solution of the fluoride can be withdrawn by the feeder. For this purpose, a sodium fluoride of larger particle size (20–40 mesh) has been produced which permits an increase in the rate of flow of water through the bed. The withdrawal rate must not exceed the rate of flow through the fluoride. A similar grain size for sodium silicofluoride would be most desirable.

Where a community is supplied with water from several different sources, the use of several small solution feeders is more economical than any attempt to bring the supplies together at a central point for treatment. Multiples of these single units may also be used at a central point. In such situations, however, a larger solution tank is required or the solution must be made up more frequently. In general, it has been more economical and convenient to use dry feeders when the daily consumption exceeds about 2.0 mgd. Solution feeders cost about $250 without auxiliary equipment.

Solution feeders of various capacities and refinements in construction can be obtained from: Graver Water Conditioning Co., N.Y.; Hills-McCanna Co., Chicago; Infilco, Inc., Chicago; Lapp Insulator Co., Leroy, N.Y.; Milton Roy Co., Philadelphia; Morse, Boulger Destructor Co., N.Y.; Omega Machine Co., Providence, R.I.; Permutit Co., Inc., N.Y.; Proportioneers, Inc., Providence, R.I.; T. Shriver and Co., Inc., Harrison, N.J.; and Wallace and Tiernan Co., Inc., Newark, N.J.

Dry feeders are of two general types: [1] volumetric feeders which deliver a measured volume of dry chemical within a given time interval; and [2] loss-in-weight feeders (gravimetric) which deliver a measured weight of chemical within a given period.

Single volumetric feeders have been used for supplies delivering between 2.0 and 5.0 mgd. Disk type volumetrics, however, can deliver up to 25 pounds of sodium fluoride per hour and roller types, up to 18 pounds per hour. If these maximum rates could be used, up to 32.6 mgd. could be treated. The volumetric feeder is utilized while resting on scales. The hopper holding the dry chemical may be fitted with a dust collecting system. These feeders cost $350 or more.

Volumetric feeders of sufficient accuracy for fluorides are manufactured by: B. F. Gump Co., Chicago; Infilco, Inc., Chicago; Jeffrey Manufacturing Co., Columbus, Ohio; Omega Machine Co., Providence, R.I.; Permutit Co., Inc., N.Y.; and Wallace and Tiernan Co., Inc., Newark, N.J.

Gravimetric, loss-in-weight, feeders have been used for the larger supplies. They are capable of feeding up to 5,000 pounds per hour, which exceeds the greatest demand on any supply in the United States. On the other hand, they are capable of feeding accurately as little as 10 pounds per hour. They are fitted with solution boxes and their built-in weighing mechanism provides a constant indication of the rate of feed. The hoppers are generally filled from the floor above and are fitted with dust collectors. Feeders of this type cost $2,000 or more, depending on size and accessories.

Slight overdosage has occasionally occurred when the solution box was flooded with fluorides. This condition results when the chemical forms an arch in the hopper. When the arch collapses, the fluorides are forced over the feeding mechanism. This disadvantage reportedly has been eliminated with the development of a locking mechanism.

Manufacturers of gravimetric feeders include: Jeffrey Manufacturing Co., Columbus, Ohio; Omega Machine Co., Providence, R.I.; and Wallace and Tiernan Co., Inc., Newark, N.J.

Small feeders are generally connected electrically with the pump motors to stop the feeder automatically when the pump stops. Manual adjustment of the rate of feed is easily made on all feeders when changes in the amount of water to be treated can be anticipated. If these changes are not predictable, feeding rates can be varied automatically, with solenoid valves actuated either by a venturi or water meter.

The mechanics of feeding fluorides (except hydrofluoric acid) are no more involved than those for other chemicals used in water purification. The major differences may be that fluorides are toxic (but so is chlorine) and that an overdosage is difficult to correct. Even with a 100 percent overdose, however, the resultant fluoride content will not exceed approximately 2.0 p.p.m. This concentration will produce no perceptible ill effects, even when continued for a week. Nevertheless, the rate of application should be maintained as constant as possible and close to the dose determined as optimum for the supply in question. Valuable information can be obtained from every community using fluoridated water. The interpretation of valid data will be contingent on a history of uninterrupted use of water containing a constant, static fluoride concentration. No valid or useful conclusions can be drawn from a study where the concentration has varied considerably and intermittently.

POINT OF APPLICATION

The point of application of the fluoride is an important factor. Almost every water supply presents a different problem, sometimes involving a compromise between what is considered the best point of application and the best place to replenish the feeder hopper. In general, fluorides are applied most effectively at that point where the risk of losing them in a subsequent treatment is least.

Untreated supplies present the fewest problems. Fluorides can be introduced either into a reservoir (if the influent rate is known), into the well directly, or into the discharge or suction side of the pump. Feeders capable of discharging solutions against any head encountered in water supply practice are available.

In treatment plants, consideration should be given to processes which have a tendency to remove fluorides. These include lime softening in the presence of magnesium; alum or sodium aluminate coagulation; feeding of bentonitic clays; and activated-carbon treatment at low pH values. As little as 10 p.p.m. alum, when added to water with 1.0 p.p.m. fluoride, will remove about 0.1 p.p.m. of the fluoride. It is therefore advisable to add fluorides after these treatment steps. If settling is prolonged and efficient, and if the filters remove relatively little material, fluorides can be added before filtration. On the other hand, if the load on the filters is considerable, fluorides should be added after filtration, generally either in the line between the filters and the clear well or in the clear well itself.

Chlorine can be added anywhere in the plant or distribution system. There are no known reactions between fluorides at 1.0 p.p.m. in water and compounds formed in water after chlorination. Fluorides at this concentration have no bactericidal effects.

SIDE EFFECTS

There are no noticeable effects of 1.0 p.p.m. fluoride on taste, odor, color, hardness or pH value. No untoward effects have been reported by bottlers, brewers, bakeries, laundries or chemical plants. At Charlotte, N.C., where the supply is relatively unmineralized (total solids, 43.7 p.p.m.; hardness, 22.8 p.p.m.; chlorides, 2.8 p.p.m.; and sulfates, 4.3 p.p.m.) cracking of ice blocks in ice manufacturing plants increased about 50 percent apparently after 1.0 p.p.m. fluoride was added (*7*). This phenomenon was completely eliminated by adding the equivalent of 20 p.p.m. ammonium chloride to the water used for ice making. No other such experience has been reported. Colorado Springs, Colo., with a water supply very similar to Charlotte's (except that 2.8 p.p.m. fluoride is naturally present), has had no trouble of this kind, but in any event, the phenomenon should be investigated.

Fluoridation will provide all or part of the fluorides needed to remove silica from boiler water. An ion-exchange process is reported to require the addition of about 1.0 p.p.m. fluoride for each 0.5 p.p.m SiO_2 removed (*8*). On the other hand, if sodium silcofluoride is used for fluoridation, about 0.5 p.p.m. SiO_2 is formed when 1.0 p.p.m. fluoride is added from this source.

After fluoridation has been started, the fluoride content of sewage almost immediately reaches the same concentration as in the water supply. This phenomenon has had no effects on any sewage treatment process.

Home softening units using the salt regeneration system will not remove the added fluorides.

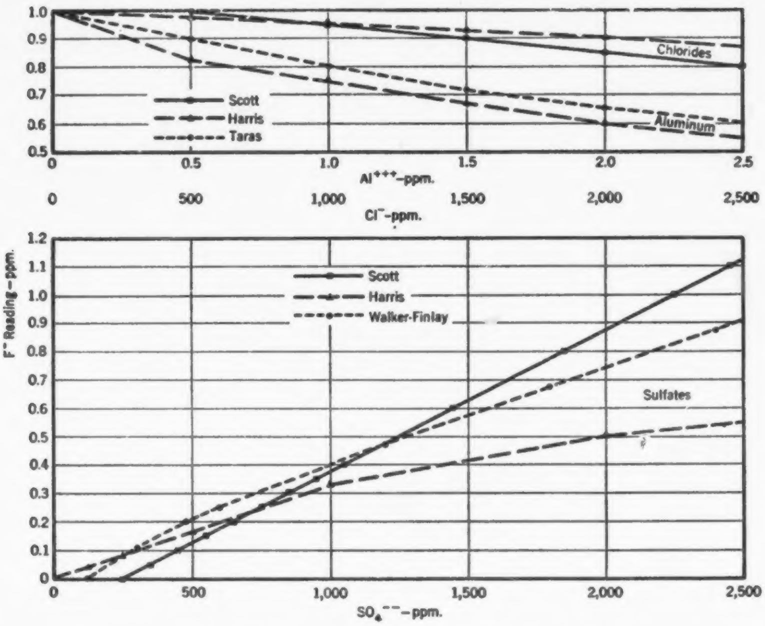


FIGURE 4.—*Range of ionic interference.*[1]

RECORDS AND SAMPLING

In even the smallest installations, daily records should be kept of water treated and amounts of fluoride fed. After it has been ascertained that the optimum fluoride concentration is reaching all points in the distribution system, sampling schedules at the plant and in the system are formulated primarily to check inaccuracies of the feeder and unexplained losses in the system. Actually, if a feeder is well maintained and adjusted, its accuracy should be well within 5 percent. This accuracy is greater than that attained by present tests for fluorides in water.

TABLE 3.—*Upper limits of ionic interference using Scott-Sanchis reagent to give maximum error of approximately 0.02 p.p.m. fluoride*

Substance	Fluoride Reading Too Low If More Than:	Fluoride Reading Too High If More Than:
	ppm.	
Chlorides	500 (2,500—Lamar)	
Aluminum*	0.10	
Alkalinity (as $CaCO_3$)	150 (400—Lamar)	
Sulfates		150 (1,000—Lamar)
Iron		0.5
Phosphates (PO_4)†		1.6–3.3
Phosphates ($(PO_3)_6$)†		0.3
Chlorine		0.02‡
Manganese		0.02‡

* Ref. 15.
† Ref. 16.
‡ Ref. 14.

The standard test for fluoride is based on the degree of decolorization of a zirconium-alizarin lake in an acid solution (*9–11*). In general, this reagent is relatively unstable, the color range is narrow, the color development requires about an hour, and many other ions in water interfere with the reading. So far, the best reagent of this type is that developed by Lamar (*12*) in 1945. It is more stable and its color range appears to be broader than is true for other reagents. Interference with other ions is considerably reduced.

As shown in figure 4, interference by other ions using the present standard method (Scott-Sanchis) is considerable. In addition, a 0.10 p.p.m. free chlorine residual considerably decolorizes the lake, producing an apparent increase in fluorides. It has been reported that water containing 1.8 p.p.m. chlorine can be dechlorinated by adding one drop of 0.1*N* sodium thiosulfate per 100 ml. of sample (*14*). Ultraviolet rays obtained either from sunlight or lamps will also dechlorinate the fluoride sample.

If the quantities of interfering ions exceed the maximums shown in table 3, the most accurate procedure is to distill the sample in perchloric or sulfuric acid (*17*). The distillate will contain all the fluorides and practically none of the interfering ions. If the excess of these ions is not too great, the sample might also be diluted with distilled water and the fluoride content of the aliquot determined in the usual way.

Setting up a series of standards—a laborious step in the fluoride determination—is no longer required in routine analysis where the accuracy desired is within 0.20 p.p.m. fluoride and the interference is negligible. Permanent color standards of glass or liquid are available and can be fitted into a comparator. The color gradations of the liquid standards in sealed tubes are based on the Lamar reagent; those of the glass standards, on the Scott-Sanchis reagent.

SAFETY MEASURES

No skeletal changes, except the mildest forms of dental fluorosis, have ever been observed in individuals who have continually used water containing 1.0 p.p.m. fluoride. Damage to the bones (osteosclerosis) has occurred after 2½ years' continuous exposure to cryolite and phosphate rock dust, whereby probably 15–25 mg. fluoride per day was inhaled (*18*). Industrial hygienists have set a threshold limit of 2.5 mg. fluoride per cubic meter of air. Present practice in water treatment plants is to obtain equipment permitting the fluoride feeder hopper to be filled no oftener than once a day. If reasonable care is used in handling the fluorides so as to produce the least dust, little danger to the operators is involved. Actually, the greatest hazard occurs in the larger plants, where the fluorides are dumped from a barrel into a hopper opening which is at floor level. Occasionally the fluoride arches in the barrel and then, suddenly emptying, throws a cloud of dust into the air. In such installations a dust exhaust system should be made part of the hopper equipment.

Hopper exhaust systems usually involve a series of socks hung in a steel enclosure opening into the top of the hopper, adjacent to the feeding door. A motor-driven fan draws air from the hopper through the socks and exhausts to the atmosphere. At Oshkosh, Wis., the suction is derived from an aspirator type water eductor. The sodium silicofluoride dust entrained in the water is carried to a sedimentation basin.

When filling hoppers, the operators should wear masks. Those of the type BM2101, equipped with a filter designed for dusts (BM2133), are satisfactory.

In smaller plants where the hopper or solution tank is replenished by means of a scoop, little or no dust is generated if the fluorides are handled carefully. Here, too, however, a mask should be worn. Rubber gloves should also be worn and the hands washed after each filling. Spilled fluorides should be wet-mopped and discarded.

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[1] References: Scott (10), Harris (15), Taras (14), Walker-Finlay (13).

The crystalline sodium fluoride, now available in a larger particle size (20–40 mesh), should reduce the dust problem to a minimum, because a very low percentage of this material contains particles small enough to form dust.

INITIATION OF FLUORIDATION

Many fluoridation projects have been started as a result of the interest created by local dental and medical societies. Many of these groups have presented their evaluations of reports containing factual and convincing proof of the benefits demonstrated at places where fluorides are naturally present and, lately, where they have been artificially added. The local councils, with or without a public hearing, but always with public support, have then authorized (sometimes by ordinance) the local water departments to proceed. A.W.W.A. official policy as published in the JOURNAL (*19*) states (sec. 4) that:

"The operating management of a water supply utility should begin the addition of fluoride in the water supply only after:

4.1. The governing body of the municipality has authorized fluoridation by ordinance, and the local and state health authorities have approved the addition of fluoride and have: [1] established reasonable maximum and minimum limits of fluoride, stated in parts per million, to be maintained in the water supply; [2] approved the type of chemical feeding equipment to be installed and used; [3] approved the installation of equipment, plant layout and methods of handling the fluoride compound to assure the safety of employees; and [4] approved the method of analysis and control to be used in determining the fluoride content of the water before and after the addition of fluoride.

4.2. The state or provincial public utility commission has released, when required, any necessary orders—for example, a method by which the utility will be reimbursed for the expense of fluoridation.

4.3. The legal counsel of the water department or company has made a finding that all necessary authorization has been given to the water utility so that legal liability for damages will be limited to negligence.

4.4. The board of directors or similar governing body of the utility has, by resolution, instructed its proper operating officers and employees to add fluoride as specifically authorized by the controlling health and municipal authorities."

Permission and advice are always obtained from the state health department, and, where applicable an opinion on liability is obtained from the state attorney general. These opinions have always reiterated the water department's liability for damage resulting from negligence. Money for fluoridation has come from the general fund or from health or water department appropriations, but usually from the health department.

SUMMARY

Before adding fluorides to the water supply, these points should be observed:

1. There is a possibility, particularly in the southern latitudes of the United States, that too much fluoride may be added, with the result that an undesirable degree of fluorosis may develop.
2. Operators should be trained to recognize the toxicity of fluorides and to handle them safely.
3. Dosages should be made as accurate and as constant as possible.
4. Complete records should be kept of the amounts of water treated and the quantities of fluoride used.
5. Daily fluoride tests of samples from the plant and distribution system should be made and recorded.

In the light of evidence obtained from places which have long used water containing the optimum fluoride concentration, and, lately, from those communities which are adding fluorides, it can be recommended that plans approved by the state health department for fluoridating the water supply should be formulated. Every delay deprives children of a substantial health benefit which may be obtained at a negligible cost.

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ENGINEERING PROBLEMS IN WATER FLUORIDATION

F. J. Maier

By and large, the solutions to the problems common to almost all fluoridation installations can be found in satisfactory answers to questions such as: How much fluoride should be added? Which fluoride compound should we use? How should we feed it? Where should it be applied? How should the operators be protected? How should we control it?

The correct answers to these questions will be obtained largely through the efforts of engineers. The design and installation of fluoridation equipment are the joint responsibility of engineers employed by the city, the state health department, consulting engineering firms, and equipment manufacturers. Plans and specifications will be prepared either by the city's own engineering staff or by consulting engineers retained by the city. These plans will be submitted to the state health department for approval. Equipment manufacturers will be governed by the specifications devised by the city and state engineers. Each of these groups will be confronted with a variety of problems which will differ in each community. The correct solution of these problems will provide a procedure comparable in its benefits to other water works practices which have contributed immeasurably to the public health.

HOW MUCH FLUORIDE SHOULD BE ADDED

A fluoride concentration of 1.0 p.p.m. is the optimum in most communities in this country. In areas where the environment stimulates an excessive water intake, it may be necessary to reduce this concentration to as little as 0.7 p.p.m. Such areas are distinguished by very high daytime temperatures and low humidities and are likely to be semi-arid or desert. They are also sparsely settled. On the other hand, where relatively little water is consumed, as in the extreme northern part of the country or in communities situated at higher altitudes, the optimum concentration may exceed 1.0 p.p.m. In some places, up to 1.5 p.p.m. may prove to be the best.

States with regions having these extremes in factors influencing water consumption are currently determining for themselves the optimum fluoride concentrations This is done by observing the dental effects of waters containing various amounts of fluorides. Nearby states, or those having a similar environment, are adopting these optimum concentrations if, for various reasons, they cannot make studies themselves.

The amount of fluoride to be applied will be the difference between the predetermined optimum amount and the concentration in the untreated water. Many water supplies have not been examined for fluorides over any extended period of time. Where such records are not available, fluoride determinations of the untreated water should be made at regular intervals during fluoridation in order to discover any variations. In cases where other treatment processes reduce the natural fluoride content, knowledge of the raw water fluoride concentration alone will not be sufficient to determine the amount to be added. Instead, the fluoride concentration should be determined at the point immediately prior to the addition of fluorides.

WHICH FLUORIDE COMPOUND SHOULD BE USED

At present, three preferred compounds are used in fluoridation: sodium fluoride, sodium silicofluoride, and hydrofluosilicic acid. Other compounds are not being seriously considered now, either because of their cost, corrosiveness, low solubility, hazards in handling, or unavailability in commercial quantities. Table 1 shows some of the characteristics of these three compounds.

Table 2 illustrates their relative order of desirability.

Chemical Cost

Based on fluoride ion content, sodium silicofluoride is the least expensive source. Sodium fluoride is about two and one-half times more costly, while 30 percent hydrofluosilicic acid is about three times as expensive. The acid can be obtained in higher concentrations, but the increase in cost is disproportionate. In order to stimulate increased production of sodium silicofluoride, the controlled price recently was increased. The increase, however, will be insufficient to affect significantly the existing advantage in cost over the other two compounds.

Solubility

If a gallon of hydrofluosilicic acid is required to fluoridate a quantity of water, then 17 gallons of a saturated solution of sodium fluoride, or 65 gallons of a saturated solution of sodium silicofluoride, will be required for the same amount of water. This inherent advantage in hydrofluosilicic acid is reflected in the lower cost of solution feeders and solution tanks. However, the advantage of the acid's high solubility is lost in the larger installations, where the other two less expensive compounds are proportioned directly with dry feeders.

Chemical Storage Space

For the same quantity of fluoride ion, sodium silicofluoride requires the least storage space; hydrofluosilicic acid the most. However, the acid is stored in tanks which might be placed outdoors or underground. Practically speaking, consideration of storage requirements as a factor in choosing a particular compound depends primarily on a comparison between costs for providing additional storage space and the price of the compounds.

Feeder Space Limitations

Hydrofluosilicic acid has been chosen in some communities where available space in small treatment plants is limited. Inasmuch as the acid can be stored in tanks at some distance from the feeder, and no solution makeup equipment is required, the use of the acid is advantageous when room is available for only the solution feeder.

Corrosiveness

Although the relative corrosiveness of the three compounds differs, equipment manufacturers provide about the same corrosion-resisting materials for solution feeders, tanks and appurtenances, regardless of the compound used. Almost the only exception is the action of hydrofluosilicic acid on ceramic crocks or glass-lined tanks (as a result of vaporization and the formation of small amounts of hydrofluoric acid on the liquid surface). However, hydrofluosilicic acid will seldom be used in such containers because it can be drawn directly from the rubber-lined drums in which it is shipped or stored.

TABLE 1.—*Characteristics of three fluoride compounds*

Chemical	*Per cent Fluoride in Commercial Chemical*	*Solubility at 25° C. g./100 ml.*	*Cost/Lb.* ¢	*Cost/Lb. Commercially Available F.* ¢
Sodium fluoride	44.4	4.05	11.5	26.0
Sodium silicofluoride	60.0	0.762	6.0	10.0
Hydrofluosilicic acid (30%)	23.7	—	7.0	30.0

TABLE 2.—*Order of desirability of three fluoride compounds*

	Sodium Fluoride	*Sodium Silicofluoride*	*Hydrofluosilicic Acid*
Cost	2.5	1.0	3.0
Solubility, gallons solution required	17.0	65.0	1.0
Storage space required	1.5	1.0	2.5
Limitations, feeder space	2.0	2.0	1.0
Corrosiveness	1.0	1.5	2.0
Incrustation in feeder	3.0	2.0	1.0
Bulk handling problems	2.0	2.0	1.0
Hazards to operators	1.0	1.5	1.0

INCRUSTATION IN FEEDING EQUIPMENT

Incrustants form occasionally in solution tanks, feeders, and feeder lines. These are essentially calcium and magnesium fluoride or silicofluoride derived from strong fluoride solutions and the hardness constituents of the water. No incrustants are formed when hydrofluosilicic acid is used because it is not ordinarily diluted with water. Incrustants formed when sodium silicofluoride is used (calcium or magnesium silicofluoride) are slightly more soluble than calcium and magnesium fluoride. The problem can be minimized by softening the solution water or applying small amounts of hexametaphosphates.

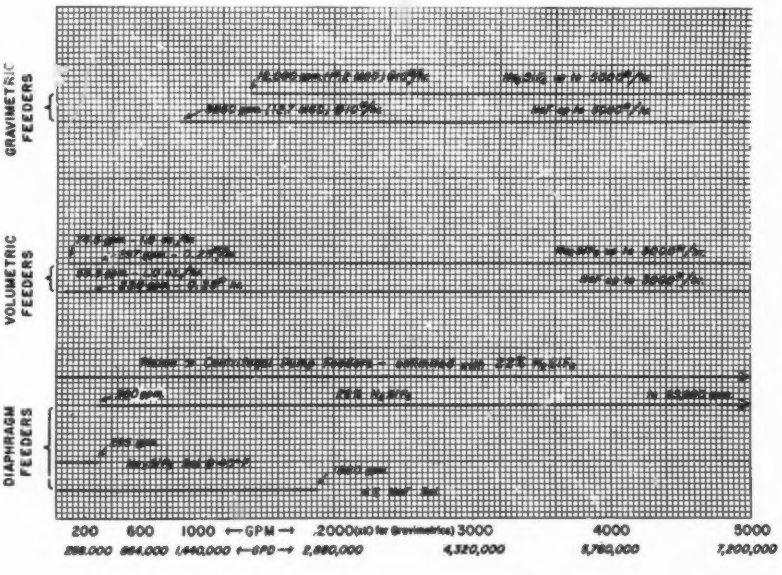
The use of softeners to reduce loss of fluorides caused by fluoride precipitation is not ordinarily justified. The savings realized over the cost of softening amount to less than $2 per year in a city of 10,000 population using water of 200 p.p.m. hardness.

There is very little additional labor involved in removing the precipitated fluorides from solution tanks, inasmuch as sodium fluoride contains insoluble material which also must be removed periodically.

BULK CHEMICAL HANDLING

Because hydrofluosilicic acid can be readily pumped, little manual labor is required at the treatment plant. At present, sodium fluoride and sodium silicofluoride can be purchased only in bags or barrels which require some manual effort to empty. For larger installations, bulk shipments may eventually become a reality, but the possible advantages of this development may be nullified by the cost of dustproof conveyors and the hazards associated with the fluorides left in the cars.

CHART 1



HAZARDS TO OPERATORS

Dust arising from the handling of powdered fluorides can be controlled by careful handling and the use of protective devices. Practically no hazards are involved in using the acid inasmuch as it is ordinarily proportioned directly from the shipping container. At some of the smaller plants, where the acid requires dilution prior to feeding, considerably more care in handling is required. No particular advantage is gained by this procedure, however, because about as much effort is required to dilute the acid as to make up the less expensive sodium fluoride solution.

HOW SHOULD FLUORIDES BE FED

The choice of a feeder depends primarily on the compound used and the amount to be fed. The rate of feed must be adjusted according to the amount of water per minute passing the point of application. For this reason, classifying feeders according to the size of the community or the number of gallons of water per day delivered by a plant is impracticable.

The capacities of various types of chemical feeders introducing 1.0 p.p.m. fluoride are shown in chart 1.

Gravimetric feeders are suitable for either sodium fluoride or sodium silicofluoride. They feed the material at a constant predetermined rate automatically controlled by the weight of the compound left in the storage hopper. The accuracy is well within 5 percent. The feeders are intended for the largest installations. A minimum of 8,900 g.p.m. should be available for treatment at the minimum rate of feed of 10 pounds of sodium fluoride per hour; 12,000 g.p.m. at the minimum rate of 10 pounds of sodium silicofluoride per hour. Machines of this type can be obtained which are capable of treating the largest water supplies in the country with 1.0 p.p.m. fluorides.

Gravimetric feeders are designed to deliver a given weight of chemical per minute while volumetric feeders proportion a particular volume of material per minute. Volumetric feeders are available in sizes comparable to gravimetrics, but inasmuch as their accuracy decreases in the larger capacities, gravimetrics should be used wherever possible. Some volumetric feeders are capable of delivering as little as one-quarter of an ounce per hour. It has been found, however, that this extremely low rate is not entirely satisfactory. The chemical tends to arch in the hopper after several weeks, probably because of its slightly hygroscopic tendencies, resulting in erratic fluoride delivery. In Wisconsin the practice now is to limit the minimum rate to about one ounce per hour. This rate corresponds to a plant delivering 55.5 g.p.m. if sodium fluoride is used and 74.5 g.p.m. when using sodium silicofluoride.

The minimum delivery rates of continuously operated diaphragm solution feeders varies from 2.5 to 12.6 ml. per minute. By intermittently operating the feeders with timed relays, deliveries can, of course, be reduced considerably further. However, on the basis of continuous operation, plants delivering less than about 50 g.p.m. should be treated with solutions of sodium fluoride of less than 4 percent strength. Saturated solutions of 4 percent sodium fluoride are continuously and automatically obtained with a saturator now available. Water is allowed to pass downward through a bed of coarse grade sodium fluoride and a saturated solution is withdrawn by the feeder from the bottom of the tank. Diaphragm solution feeders can be used with saturated sodium fluoride solutions at plants delivering 50 g.p.m. and up to 1,880 g.p.m. However, in the upper reaches of this range, sodium silicofluoride fed with volumetric feeders would be more economical. In general, where capacities of solution feeders for 4 percent sodium fluoride and volumetric feeders for sodium silicofluoride overlap, the deciding factor may well be the savings accruing over the estimated life of the feeder based on the delivered price of the two compounds.

Solution feeding of sodium silicofluoride, while theoretically possible, may prove impracticable from the standpoint of preparing accurate solutions, particularly when solution water temperatures vary.

Undiluted 22 percent hydrofluosilicic acid cannot be used in water supplies delivering less than 150 g.p.m. when the minimum feeder rate is 2.5 ml. per minute. Solution feeders are available for feeding this acid in any higher quantity against any pressure encountered in water supply practice. Piston-type solution feeders of considerably lower capacities have been recently announced, but none so far have been used for this purpose.

Where Should Fluorides Be Applied

An important consideration in fluoridation is the determination of the point in the water system at which fluorides should be applied.

Generally, untreated water supplies present the fewest problems. Inasmuch as these supplies are derived principally from wells, the fluorides are injected into the discharge side of the well pump. Other factors being equal, the preferred location should be chosen from the point of view of absence of excessive pressures, convenience of replenishing the fluoride solutions and ease in measuring the quantity of water treated. Consideration also should be given to locations where back-siphonage of the fluoride solution through the feeder is most improbable.

In systems where the water is otherwise treated, the principal considerations in choosing the point of application are the quantities of fluorides which will be lost during subsequent treatment processes and the convenience in replenishing the feeder hoppers. In some few instances, it is economically justifiable to realize some loss of fluoride. Where transportation, storage or handling costs can be substantially reduced by adding fluorides to the raw or partially treated water at a more accessible point, the loss of fluorides through the plant may be small enough, in some cases, to realize a substantial saving.

How to Protect Operators

The hazards inherent in the handling of fluorides by water works personnel can be eliminated by utilizing four safeguards: choice of fluoride compound; proper handling procedures; personal protective devices; and dust collecting apparatus.

The inhalation of fluoride dust is the principal hazard to operators. This can be minimized considerably by using a recently developed crystalline sodium fluoride which contains practically no particles small enough to form dust. Careful handling of the powdered compounds, either while emptying barrels or when transferring small quantities from the shipping containers to solution tanks, is most important. Spilt fluorides should be removed by wet mopping. Masks should be worn to protect operators in the event dust is accidentally generated. These should be of the type classified by the Bureau of Mines as approved for "all dust" or "toxic dust." Replacement filters should be of the type bearing the same approval classification. Rubber gloves should be worn because fluorides are a skin irritant. Where large quantities of fluorides are dumped into feeder hoppers from barrels or bags, blower-operated dust collectors should be installed in the hoppers. Pneumatic conveyors should be vacuum-operated.

How to Control the Fluoride Concentration

The most important consideration in assuring a constant, invariable fluoride concentration is the selection of accurate feeding equipment. If good feeders are properly operated and maintained, their accuracy can be assured indefinitely.

In order to check this accuracy and to make certain that the same fluoride concentration is reaching all parts of the distribution system, two controls should be used. One requires periodic fluoride determinations of representative water samples; the other, a determination of the amount of fluoride used and the amount of water treated over a given period. The latter control is difficult where fluoride saturators are used, because accurate measurements of the amounts of fluorides used cannot be obtained except during a prolonged period. The saturator must be replenished well before the previously added fluorides are dissolved, thus leaving an unknown amount. This disadvantage, however, is minimized by the definite superiority of the saturator in eliminating errors in preparing unsaturated fluoride solutions.

The fluorides in water should be determined at least once a day, and, in the larger plants, several times a day. The present test is based on the decolorization of a zirconium-alizarin lake in an acid solution. The reagent is not stable, the color development requires about an hour, the range is narrow, and many contaminating ions in water interfere with the reading. While the interference of most ions is linear, the effect of aluminum, phosphates, and chlorine varies with the actual fluoride concentration. When significant quantities of interfering ions are present, distillation of the sample is almost obligatory.

SUMMARY

Engineers concerned with the design of the average fluoridation installation will be obligated to provide satisfactory solutions to these questions which almost invariably arise.

1. How much fluoride should be added?
2. Which fluoride compound should be used?
3. How should fluorides be fed?
4. Where should fluorides be applied?
5. How should operators be protected?
6. How should the fluoride concentrations be controlled?

If the problems posed by these questions are effectively solved, the community will not only benefit from the considerable reduction in caries incidence, but will obtain this advantage in a way that is safest and most economical.

DILUTION OF FLUOSILICIC ACID

ERVIN BELLACK and F. J. MAIER

When undiluted fluosilicic acid is fed directly into water to produce a concentration of approximately 1.0 p.p.m. fluoride, no particular difficulties are encountered. When attempts have been made to use this acid at smaller plants, however, where limitations of the chemical feeders have required that the acid be diluted prior to feeding, considerable difficulties have been experienced. These difficulties have arisen when the acids are diluted in the order of more than 10 parts of water to 1 of acid, resulting in the formation of a visible, insoluble precipitate which clogs the feeder and its appurtenances, valves, pumps, and orifices. The composition of this precipitate has been investigated and it was found to be finely divided silica.

The precipitate forms regardless of the nature of the water used for dilution of the acid, and distilled water causes it to form as readily as does water containing more than 2,000 p.p.m. of dissolved solids. The amount of dilution is critical, however. Beyond a ratio of about twenty to one, when an acid containing the greatest silica content obtainable is used, no precipitate is formed. Not all of the commercially available acids exhibit this precipitate-forming characteristic, however.

METHODS OF PRODUCTION

Two methods are used for producing this acid. The more common involves the absorption by water of silicon tetrafluoride, produced by the acidification of phosphate rock. The fluosilicic acid produced by this process contains appreciable and varying amounts of free silica.

The other method is essentially a controlled reaction of silica in aqueous hydrofluoric acid, and the fluosilicic acid thus formed is of higher strength and, being practically free of colloidal silica, will not form a precipitate on dilution. Because this method is more expensive, however, most of the fluosilicic acid used for water fluoridation is produced by the former method, resulting in greater amounts of free silica.

USE OF HYDROFLUORIC ACID

To prevent the formation of objectionable precipitate, it was thought possible either to stabilize the colloidal suspension or remove the excess silica by physical or chemical means. To this end, several additives were tried, but only strong acids gave any measure of success, and, of these, hydrofluoric acid seemed most promising. Hydrofluoric acid not only dissolves the colloidal silica, but is entirely compatible with the fluosilicic acid solution equilibria:

$$H_2SiF_6 = 2H^+ SiF_6^{--}$$
$$SiF_6^{--} + 2H_2O = SiO_2 + 4H^+ + 6F^-$$

CONCLUSION

With the commercial acids available, the optimum ratio for the elimination of precipitation was found to be 0.2 ml. of 48 percent hydrofluoric acid to 10.0 ml. of 30 percent fluosilicic acid. The commercial fluosilicic acid could then be fortified on the basis of 1 gallon of hydrofluoric acid to 50 gallons of fluosilicic acid. The addition of this amount of hydrofluoric acid would alter the corrosive characteristics of the mixture very little and the increased chemical and handling costs would be offset by the higher fluoride ion concentration in the resulting dilution. Optimum ratios for acids having different colloidal-silica concentrations would necessarily have to be determined by the acid manufacturers. In the interests of safety and maximum effectiveness, these manufacturers should also stabilize the acid at their plants by adding the optimum quantities of hydrofluoric acid.

FLUORSPAR FOR FLUORIDATION

FRANZ J. MAIER and ERVIN BELLACK

The principal items of cost in fluoridating public water supplies are the chemicals, the feeding equipment, and labor; of these, the chemical costs are by far the greatest. Consequently, if the cost of chemicals were reduced, the fluoridation procedure would be an even smaller factor economically. The greatest reduction of such costs can be achieved by using fluorspar, the cheapest of all commercial fluorides. The least costly fluoride compound now being used for fluoridation, sodium silicofluoride, costs three times as much as fluorspar.

Except for the silicofluorides, which are obtained as a byproduct of the phosphate industry, fluorspar is the basic raw material for all other compounds containing fluoride. Fluorspar is a nonmetallic mineral which contains a sufficient amount of fluorite (CaF_2) to make it commercially valuable. Fluorite contains 51.1 percent calcium and 48.9 percent fluorine. Industrial grades of fluorspar, prepared for the consumer, normally contain from 85 to more than 98 percent fluorite. The untreated ores may contain as little as 30 percent fluorite which must be increased by methods appropriate to the ultimate use of the fluorspar. Fluorite is a lustrous, glasslike mineral, generally translucent to transparent. It may be clear and colorless, or range in color from blue to violet, amethyst, purple-green, and yellow. It commonly occurs in masses of very pure crystalline material, and is found with calcite, barite, quartz, and galena, from which it must be separated. The largest deposits are found in sedimentary rocks at shallow depths.

OCCURRENCE

Fluorspar occurs on every continent, and there is substantial commercial production in Argentina, Australia, Canada, China, Korea, England, France, Germany, Italy, Mexico, Newfoundland, Russia, Spain, Tunisia, Union of South Africa, and the United States. The largest deposits so far discovered are in southern Illinois and northwestern Kentucky, where the fluorspar area covers about 700 square miles. Colorado and New Mexico are the principal Western States producing fluorspar.

The first recorded use of fluorspar in the United States occurred in 1823 when a small amount from Shawneetown, Ill., was mixed with sulfuric acid to produce hydrofluoric acid. While the value of fluorspar as a flux was known in 1529, it was not until 1837 that some fluorspar mined near Trumball, Conn., was used in the United States with iron pyrite in the smelting of copper ores. Production was low until the value of fluorspar as a flux in the production of open-hearth steel was discovered. Imports and domestic production rose to over 300,000 tons per year prior to World War II. Since then, normal consumption has averaged about 500,000 tons per year.

Fluorspar ore, as mined, is generally intermixed with many other rocks which must be separated from it before commercial distribution. The methods of separation are standard ore-dressing techniques, including washing, screening, gravity separation by jigs and tables, and flotation. Some processes are very simple, involving only crushing and screening—as in the preparation of fluorspar as a flux for steel making—or very complicated—as in preparing it for use in ceramics or acid making (*1*).

Fluorspar must be high in calcium fluoride and low in silica for manufacture of hydrofluoric acid and fluoride compounds. Very little fluorspar can meet the specifications for chemical grade material without special purification processes—usually flotation. A typical process of such ore beneficiation would be as follows: The ore is fed through a crushing system to reduce the size to about ⅜–1½. A ball mill, in conjunction with a cross-flow or spiral classifier, reduces this size to between 35 and 200 mesh. Most ores require the removal of zinc or lead sulfides by flotation. Soda ash is added to the flotation circuit to hold the pH at 8.5–10. Other reagents usually include a frother, oleic acid (for collector purposes), and quebracho (a calcite depressor). Three to seven cleanings or "passes" are required to produce acid grade fluorspar. Final flotation concentrate is pumped to a thickener from which the pulp (50–60 percent solids) is pumped to a rotary-drum filter. The filter cake goes to a rotary dryer which reduces moisture content to 1 percent (*2*).

COMMERCIAL FLUORSPAR

Three principal grades of fluorspar are commercially available. Metallurgical fluorspar for steel making (55 percent of the total) should contain about 85 percent fluorite and not more than 5 percent silica. Acid grade (about 28 percent of the total) is usually at least 98 percent fluorite and less than 1 percent silica. The ceramic grade (an intermediate one) requires about a 95 percent purity with no more than 2.5 percent silica. At the present time, the respective fob domestic-mines prices are $38, $48 and $43 per ton (*3*). The present minimum cost of fluoridation (using sodium silicofluoride) is three times what it would be if fluorspar, at $40 per ton, were used.

In 1950 it was suggested that calcium fluoride, being the cheapest commercial source of fluoride, could be used to advantage in larger water plants where it might be fed directly into the water (*4*). Acting on this information, at least one water plant contemplated using calcium fluoride and completed a series of experiments to determine the behavior of the compound in practice (*5*). It was found that the handbook value for fluorspar solubility (0.0016 percent) was very misleading. Much of the material was not even wetted by water (because of the action of the flotation reagents), much less dissolved. Further experiments showed that a large excess of calcium fluoride, in the form of fluorspar previously calcined, plus violent stirring and long retention time, were necessary before even a 1-mg/l fluoride solution could be obtained. It was apparent that some means of putting calcium fluoride into solution was needed before the economic advantage of its use could be realized.

Although fluorspar has very low solubility in water, "it is slightly soluble in cold, strong acids and quite readily soluble in solution of aluminum salts" (*6*). This gave two possibilities for achieving solutions of calcium fluoride in a practicable manner. The first, dissolving in acid, was quickly ruled out because the amount of the cheapest strong acid (sulfuric acid) necessary to dissolve the fluorspar would raise the cost of the resulting fluoride solution to that of sodium silicofluoride and, in addition, would complicate the preparation of the solution in a water plant. The second possibility, a solution of aluminum salts, is much more practical, because one of the more commonly used chemicals in water plants is filter alum.

In a laboratory experiment, weighed amounts of finely ground fluorspar were added to alum solutions of varying concentrations. The mixtures were stirred and samples of supernatant liquid were withdrawn regularly for fluoride analysis. After 24 hours, no further quantities of fluorspar were dissolved, and the fluoride ion concentration approximated 0.1 of the alum concentration. In other words, a 10 percent alum solution contained 1 percent fluoride, or 10,000 mg/l as F^-. The same ratio held for the range of 1–30 percent alum, giving fluoride-ion concentrations of 1,000 to 30,000 mg/l.

It was found later that with an excess of fluorspar present, and with vigorous stirring, saturated fluoride solution of strengths proportional to the alum concentrations could be obtained in 2 hours. This was much more practicable and led the way to the development of a dissolver.

MODEL DISSOLVER

The first workable model of the dissolver consisted of a round, cone-bottomed glass container with an inverted glass funnel suspended within. A stirring paddle which rotated in the center of the funnel was driven by a vertical shaft inserted through the stem of the funnel. The funnel and the cone bottom provided a means for stilling and contact similar to that of sludge blanket type softening and clarification equipment. With this arrangement, practically all the undissolved solids remained in the cone, while the alum solution passed downward through the funnel stem, dissolved the fluorspar, and rose around the outside of the funnel as a clear solution saturated with fluoride ion.

The placement of the funnel relative to the stirring paddle proved to be critical, as was the design of the paddle itself. With the paddle considerably below the funnel, solids were circulated all the way to the top of the container, and came over with the fluoride solution. A conventional propeller produced a vertical thrust and gave a similar effect. A flat-bladed impeller located just within the funnel solved both these difficulties. The funnel and stirrer assembly could then be lowered or raised as a unit to reduce carry-over of solids to a minimum. Considerable experimentation with flow rates through the dissolver showed that the fluoride concentration could be varied within certain limits, but best results were obtained when the device was operated slowly enough to provide a minimum retention time of about 4 hours to ensure saturation.

PILOT MODEL

On the basis of the successful operation of the laboratory model dissolver, a pilot model large enough for a small community water supply was designed. Based on proportions obtained from the laboratory model, the 2-foot diameter,

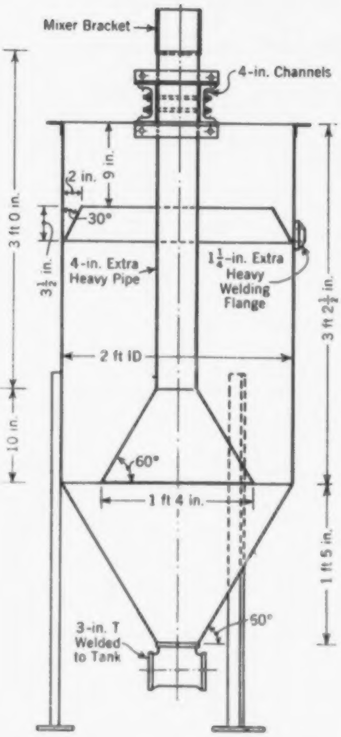


Figure 1.—*Diagram of pilot model of dissolver.*

stainless-steel pilot model was 4.5 feet high with a 60-deg. cone bottom. Its liquid capacity was about 75 gallons. A circular weir around the inside of the tank provided means for liquid takeoff. The central tube, corresponding to the stem of the funnel, was made of extra-heavy, stainless-steel pipe so that it could be fixed securely in position to provide a support for the mixer. Vertical adjustment of the inner cone was accomplished by a system of channels and angles bolted in the position shown in figure 1. A 3-inch tee was welded to the bottom of the tank to provide a cleanout for sludge. The entire tank assembly was supported by angles welded to the sides.

To prepare the dissolver for operation, the tee at the bottom was fitted with two plug valves; one for flushing water and the other for removing the sludge. The opening from the circular weir was fitted with a check valve and an ejector for removing the effluent. The 6-inch diameter, stainless-steel, flat-bladed turbine impeller was rotated at 383 r.p.m. within the inverted funnel by means of a ¼-h.p. gear-driven motor. The funnel and tube assembly were bolted in position so that the bottom of the funnel was about 3 inches above the cone part of the tank.

In order to prove its practicability, the dissolver was installed in the filtration plant at Bel Air, Md., on July 6, 1956, and has been running almost continuously since then. Arrangements were made with the owners of the plant and the Maryland State Department of Health to install the dissolver in place of a sodium fluoride solution feeder.

At this plant, water is pumped at a constant rate of 500 g.p.m., with the raw-water pump operated manually according to the water level in the filters. En route to the settling tank, alum, soda ash, fluoride, and hypochlorite solutions are added. The flocculation zone in the settling tank provides a detention period of 5 minutes, and settling requires 4½ hours. The settled water flows by gravity to two steel, 15-foot diameter sand filters and thence to a 37,000-gallon steel clear well. A high-lift pump of 260 g.p.m. capacity discharges to the distribution system and to the two reservoirs which ride on the system. Because fluorides were added (and still are) along with alum, some loss of fluorides occurred when the alum doses were high. Such fluoride losses continued when fluorspar replaced sodium fluoride.

Dissolver in Use

The dissolver was placed near the crock used for making the alum solution (fig. 2), and was charged with 300 pounds of fluorspar in an alum solution. The alum crock was charged with 50 pounds of alum in 50 gallons of water. This produced an alum solution of approximately 11 percent strength. By adjusting the alum feeder supplying the dissolver, 1 mg/l fluoride could be fed to the raw water along with about 10 mg/l of alum. Inasmuch as the raw-water pump was operated intermittently, the mixer motor, alum feeder, and the solenoid valve (on the water line to the eductor) were electrically connected to the pump motor starter. This connection eliminated the possibility of feeding fluorides when the raw-water pump was idle.

The dissolver can fluoridate up to approximately 6 mil gal of water per 100 pounds of 97 percent fluorspar if the fluoride concentration is maintained at 1 mg/l and no coagulation losses are

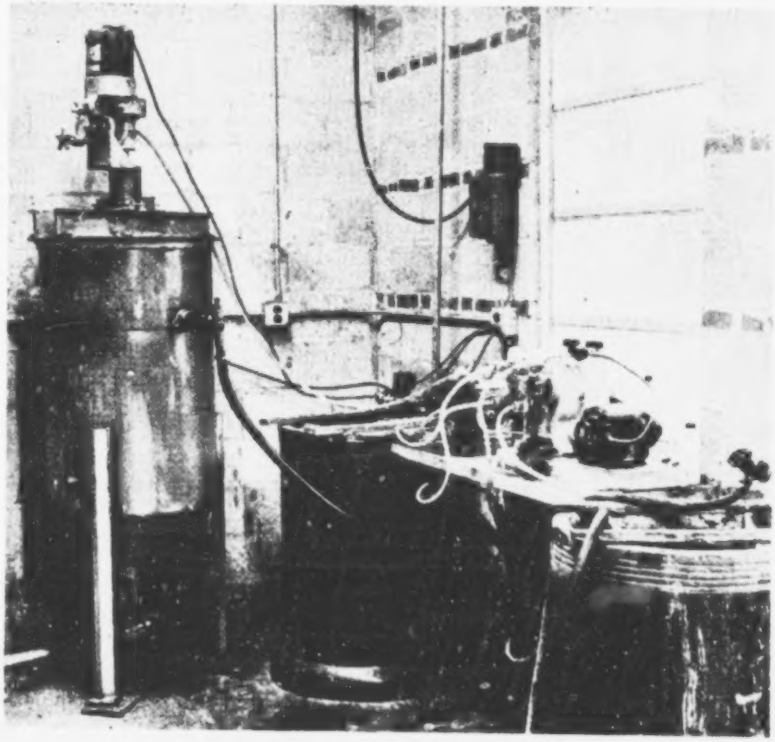


Figure 2.—*Fluospar dissolver in use. Adjacent to the dissolver, at left, are the alum crocks used in conjunction with the dissolver.*

experienced. The addition of 100 pounds of fluorspar after 5–6 mil gal of water are treated will ensure a constant fluoride level, because a reserve of approximately 200 pounds of fluorspar remains in the dissolver. In recharging the dissolver, 100 pounds of fluorspar are ordinarily dumped into the alum solution with the stirrer running. If the fluorspar contains no flotation reagents, it sinks immediately; if flotation reagents are present, a thick foam forms on the surface of the alum solution. This foam retains a considerable amount of fluorspar, much of which does not subsequently dissolve, and is lost. The quantity of foam can be reduced by premixing small portions of fluorspar with water and pouring the mixture into the dissolver.

A clear effluent can be obtained from this dissolver at withdrawal rates up to 555 ml. per minute. This is equivalent to treating 2.5 m.g.d. at 1 mg/l F^-. The rate can be increased to 666 ml. per minute (equivalent to 3 m.g.d. at 1 mg/l F^-), but the effluent becomes cloudy. This cloudiness is caused by calcium sulfate particles formed by metathesis with the fluorspar and alum. No visible calcium fluoride particles are suspended at the lower rate.

The calcium sulfate produced (theoretically 1.7 pounds of calcium sulfate is formed from 1 pound of fluorspar) must be removed periodically. Each time 100 pounds of fluorspar is added to the dissolver, an equivalent volume of calcium sulfate in alum solution is removed by means of a siphon. The level of the siphon suction must be determined by trial if fluorspar removal is to be avoided. Based on the analysis of samples of sludge from different levels, it appears that the success of this process of dissolving fluorspar is based on the continuous subsidence of the fluorspar through the layer of calcium sulfate previously formed. Samples of this sludge contained 50 percent calcium sulfate, 10 percent alum and fluorides, and 40 percent water.

CONCLUSION

At the time of writing the dissolver had been operated at Bel Air for 3 months. During this time, when the alum dosages were maintained at 10 mg/l (during periods when the source was clear), no detectable fluoride losses were encountered. When turbidity of the raw water increased, the alum dosages were correspondingly raised by operating a supplemental alum solution feeder. When the alum dose reached 100 mg/l, the fluoride loss (sedimentation tank to clear well) was about 33 per cent. To compensate for this, the dissolver feeder rate was increased, which also increased the amount of alum being fed together with the fluoride. This permitted a decrease in the supplemental alum feeder setting.

It is recognized that the dissolver described could be improved with further investigation, and that the design as shown is not the only method by which the results accomplished could be attained. The principal used for dissolving fluorspar for water fluoridation, however, is believed practical, and merits further study.

ACKNOWLEDGMENTS

The help and encouragement of Clyde Albert, water superintendent at Bel Air, and Martin Flentje, director of research, American Water Works Service Company, Philadelphia, are gratefully acknowledged. The authors also thank Gil Montgomery of Minerva Oil Company and Henry Siegmann of Continental Ore Corporation for providing samples of fluorspar and technical data on its use. The chemical feeder used was on loan from Proportioneers, Incorporated.

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ADVANCES IN THE USE OF FLUORSPAR FOR FLUORIDATION

F. J. Maier

In 1945, sodium fluoride was first added to potable water in controlled amounts to produce a level of 1.0 p.p.m. fluoride. Since then, fluoridation has been amply demonstrated to be beneficial in reducing dental caries and to produce no harmful effects. At present, more than 34 million persons living in 1,775 United States communities are using an artificially fluoridated water supply.

Inasmuch as fluorspar is by far the least expensive commercially available fluoride compound, it would be a distinct advantage if it could be used for water treatment. Unfortunately, because of its insolubility in water, it has long been impossible to use it. Methods of using fluorspar in water treatment have been developed, however, and are described in what follows.

SOURCES AND PROPERTIES

Fluorspar is the principal source of commercial fluoride compounds, although known deposits of phosphate rocks (apatites) may contain more fluorides. Certain technical and economic problems must still be overcome if apatites are to compete successfully with fluorspar as a source of fluorides. Cryolites have an even higher fluoride content, but deposits of these minerals are relatively very small.

Fluorspar is a mineral containing varying amounts of calcium fluoride. Pure calcium fluoride contains 51.1 percent calcium and 48.9 percent fluoride. Raw fluorspar may contain as little as 30 percent calcium fluoride, but such a low grade cannot be used commercially without some refining. Commercial grades of fluorspar usually contain 85–98 percent calcium fluoride. Pure fluorspar is a lustrous, glasslike material almost always translucent or transparent, and may be clear or any color of the spectrum. Deposits often occur in masses of very pure crystalline material mixed with pieces of various contaminants. Deposits occur in every part of the world, and there is substantial commercial production in several countries. The largest deposits so far found are in southern Illinois and northeastern Kentucky, an area comprising 700 sq. mi., where there is a reserve of fluorspar sufficient to supply the needs of water plants for the next 20 years at the present rate of consumption. Smaller deposits are found in some of the western states, notably Colorado, Nevada, Montana, and New Mexico.

Fluorspar ore is usually mixed with many other rocks which must be separated from it before it is commercially acceptable, and must be subjected to elaborate separation techniques which include washing, screening, gravity separation by jigs and tables and flotation. In addition to the usual harmless impurities—calcium carbonate, silicon dioxide, clay, and sand—there are also harmful impurities, including barium sulfate, lead sulfide, zinc sulfide, iron sulfide, and many other compounds of lead, zinc, and iron. The sulfides are often found in such quantity that they can be economically recovered from the ore.

Fluorspar must be low in silica and most other impurities for the manufacture of hydrofluoric acid and other fluoride compounds. This degree of purity can be obtained only by a special purification process, which usually includes flotation.

Uses in Water Treatment

Fluorspar is used in water fluoridation in either of two ways: (*1*) the mineral is dissolved at the water plant and fed as a solution, or (*2*) it provides the principal raw material in the manufacture of the various fluoride compounds that can be fed directly either as a liquid or solid. The primary compound manufactured from fluorspar is hydrofluoric acid. This is used in the preparation of most fluoride salts. This acid is obtained by acidulation of fluorspar with sulfuric acid:

$$CaF_2 + H_2SO_4 \rightarrow CaSO_4 + 2HF$$

Hydrofluoric acid could be used (and was once used in at least one municipal water plant) directly, but it is usually too difficult to handle in water plants. Instead, the acid usually is used to make other compounds, which can be used directly. These include sodium fluoride.

ADVANTAGES OF FLUORSPAR

It is by far more economical to dissolve the fluorspar at the plant, because fluorspar is less expensive than any other available compound. Sodium silicofluoride is three times as expensive; ammonium silicofluoride is three and one-half times as expensive; sodium fluoride is six and one-half times as expensive; magnesium silicofluoride is seven times as expensive; and hydrofluosilicic acid is nine times as expensive.

The use of fluorspar at the plant makes necessary a means for dissolving calcium fluoride, which is almost insoluble in water. It has long been known, however (*1*), that calcium fluoride is soluble in strong acids or in solutions of aluminum compounds. It is more expensive to dissolve fluorspar in strong acids than it is to use the commercially prepared compounds, however. The use of solutions containing aluminum compounds is considerably more practical, in that one of the chemicals routinely employed in water plants as a coagulant is alum (aluminum sulfate) and it has been found that by varying the concentration of alum in solution, any desired quantity of fluoride can be obtained from fluorspar.

Direct Use

In a saturated fluoride solution, the fluoride concentration is about one tenth the alum concentration—that is, a 10 percent alum solution contains approximately 1 percent fluoride, and a 30 percent alum solution produces a saturated fluoride solution containing approximately 3 percent fluoride (30,000 p.p.m. fluoride ion). Any fluoride strength between these extremes can be obtained by changing the concentration of alum.

Before a saturated fluorspar solution can be attained with alum, the following conditions must be satisfied:

1. Sufficient time must be provided for dissolving—usually about 2 hours at ordinary room temperatures (above 60° F.).
2. An excess of fluorspar must always be present in the alum solution.
3. Vigorous stirring must be continued during the dissolving period.

BEL AIR PLANT

All of these conditions can be met if the dissolving tank is properly designed. The first such tank, (*2*) installed at the Bel Air, Md., water plant in July 1956, is 2 feet in diameter and 4½ feet high. Its capacity is about 75 gal. A circular weir around the inside of the tank provides a means for collecting and removing the dissolved fluoride and alum solution, which rises through the bed of fluorspar in the bottom of the tank. A central tube of stainless-steel pipe is fixed in the middle of the tank to support the mixer, and a cone is attached to the bottom of the pipe.

The device is placed near the source of alum solution. A small solution feeder previously used for feeding alum solution into the raw water is so arranged that it discharges into the central tube of the dissolver instead. The dissolver is charged with about 300 lb. of fluorspar and the alum solution is made up by dissolving about 50 lb. of alum in 50 gal. of water, forming an alum solution of approximately 11 percent strength. By adjusting the alum feed rate, 1.0 p.p.m. fluoride can be fed into the raw water with 10 p.p.m. alum. At these ratios, the dissolver can fluoridate up to 6 mil gal of water for each 100 lb. of fluorspar. Ater 6 mil gal has been treated, an additional 100 lb. of fluorspar is added to the tank to insure a constant fluoride level and provide a reserve. A clear effluent can be obtained from a dissolver of this size at upflow rates of 555 ml/min. This is equivalent to treating 2.3 mgd (1,610 g.p.m.) with 1.0 p.p.m. fluoride. The rate can be increased to 666 ml/min, which is sufficient to treat 2.8 mgd (1,940 g.p.m.) at 1.0 p.p.m. fluoride, but the effluent from the tank is slightly cloudy. The cloudiness of the solution is caused by calcium sulfate (gypsum) particles formed by the reaction between calcium fluoride and aluminum sulfate. At the lower rate (less than 555 ml/min) no visible calcium sulfate particles are suspended.

Calcium sulfate is produced at the rate of 1.7 lb. for each pound of fluorspar dissolved in an alum solution. This must be removed from the tank whenever more fluorspar is added; otherwise the tank would fill up with solids. At Bel Air this removal is accomplished by siphoning off a slurry of calcium sulfate until enough room is provided for an additional 100 lb. of fluorspar.

The equipment designed for Bel Air is considered to be particularly adaptable for smaller water plants to provide a fluoride residual of 1.0 p.p.m. at rates of delivery of less than 1,600 g.p.m. (2.3 m.g.d.). Treatment plants of this size are usually equipped with proportionate solution feeders for alum solutions. In such instances, two alum solution feeders must be available. The alum feeder that serves the fluorspar dissolver is adjusted to deliver the minimum alum concentration necessary for coagulation (at Bel Air this was 10 p.p.m.). Any additional alum required must be fed with another solution feeder which discharges directly into the raw water—not into the fluorspar dissolver. If a reduction in fluoride dose is desired, it may be accomplished by either of two methods. One is to control the amount of alum solution fed, keeping the concentration constant; the other method, which is more cumbersome, requires a reduction in the one alum concentration (thereby changing the amount of fluorspar dissolved and discharged) and a proportionate increase in the quantity of the supplemental alum solution fed in order to supply the same total alum dose.

Suitability for Larger Plants

One possible disadvantage of the Bel Air equipment in larger plants would be the difficulty of removing the larger quantities of calcium sulfate formed in the dissolver. The small quantities produced at Bel Air are disposed of easily by siphoning them from the dissolver into a floor drain and thence into the nearby creek. This chore must be performed only about once a month. It would be a laborious process if large quantities were involved. Dumping 100-lb. bags of fluorspar into the dissolver once a month is not particularly difficult, but it could be a serious problem if more frequent additions and larger quantities were necessary. For these reasons the design of the fluorspar dissolved at Bel Air is not ideally suited for larger treatment plants. Accordingly, a revision was undertaken which was intended to overcome these objections and which could be scaled upward to accommodate almost any size of plant.

Unit for Large Plants

An opportunity to build, install, and test an installation for larger plants was afforded by a leading producer of fluorspar, who contributed the apparatus to the water utility serving Rosiclare and Elizabethtown, Ill., and sufficient fluorspar to fluoridate the supply for the next 5 years. Drawings and specifications of the installation had to be reviewed and approved by the Illinois State Health Department. The city authorities voted to fluoridate their water and agreed to have the water plant employees trained to maintain and operate the equipment. The fluorspar dissolver for this plant is shown in figure 1 and 2.

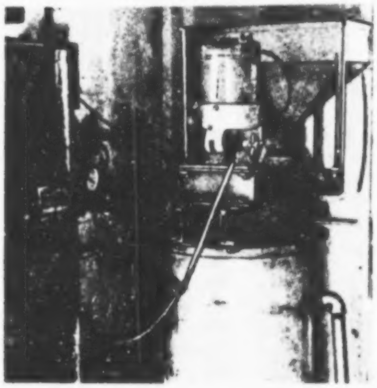

Figure 1.—*Fluorspar dissolver. A schematic drawing of the complete installation is given in figure 2.*

Three fundamental changes were incorporated in the design:

1. An existing volumetric dry feeder for alum was the source of the alum solution and a constant portion of this solution was metered into the fluorspar dissolver. Consequently, in order to change the fluoride dose, provision had to be made for controlling the alum concentration from the dry-feeder dissolving chamber. This was done by careful regulation of the amount of water entering the dissolving chamber of the alum feeder. On the other hand, if a change in the quality of the raw water made it necessary to adjust the alum dose, leaving the fluoride level the same, the alum feeder was reset and the amount of water flowing to the dissolving chamber was changed to produce the same alum solution strength as before.

2. The rate at which alum solution is introduced into the dissolving chamber was selected and fixed experimentally from observations of the hydraulic separation of fluorspar and calcium sulfate. If this rate is constantly maintained for a dissolver of a given surface area, the amount of calcium sulfate discharged should equal the amount formed. The calcium sulfate would, of course, be discharged into the water along with the alum solution and dissolved fluoride. The addition of calcium sulfate into the water presents no difficulties in subsequent treatment processes; it is, in fact, a slight advantage in that it provides additional nuclei for floc formation.

3. A volumetric dry feeder is provided to feed fluorspar continuously. This is done in order to facilitate the addition of fluorspar to the dissolver and to provide a constant concentration of dissolved solids in the dissolver. The latter is important because the time required to raise the solids "blanket" to the level of the weir is directly proportional to the distance it is raised. Consequently, in fixing the optimum solids-contact rate for alum solution for a comparatively constant period of operation, the same distance between the weir and the solids blanket must be maintained when the dissolver is not operating. This is done by equating the amounts of fluorspar added and dissolved, and the amounts of calcium sulfate formed and discharged.

In order to accomplish these three objectives, several pieces of equipment were added to the Rosiclare plant (fig. 2). The existing volumetric feeder was mounted on a larger (55-gal.) dissolving chamber, and the entire assembly was mounted on a platform scales. In order to insure complete dissolving of the alum, the water jet agitators are supplemented by an electric mixer. In addition, the water entering the dissolving chamber is controlled by the following devices installed on the water line: a shutoff valve, a solenoid valve, a strainer, pressure regulator, pressure gage, water meter, needle valve, rate of flow indicator, vacuum breaker, and flexible connector. Most of this apparatus is included to control the flow of water into the dissolver as constantly and as accurately as possible.

On the outlet from the alum-dissolving chamber, a flow splitter, consisting of two 3-in. tees in series, permits part of the effluent to flow directly into the raw-water channel and part to flow to the intake of the suction line of the alum solution feeder. The latter delivers a constant quantity of alum solution into the central column of the fluorspar dissolver.

The fluorspar dissolver is fitted with an inner cone housing an electrically driven turbine mixer. The central cone also provides a means for introducing fluorspar into the dissolver from a vibratory volumetric feeder mounted on top of the dissolving tank. The feeder is fitted with a hopper, which holds 100 lb. of fluorspar, and a rheostat for changing the rate of feed.

Operation of Large Unit

The plant is started by turning on the raw-water pump, which delivers 460–570 g.p.m. (depending on the river stage) of Ohio River water into the mixing channel. There the alum and dissolved fluorspar are added, together with a small amount of lime, for pH control. The treated water passes a series of flocculators, and the subsequently formed floc is settled out in a 180,000-gal. sedimentation basin. From there the water flows through two 58-sq ft filters into a 60,000-gal. clear well. High-lift pumps raise the treated water to a 100,000-gal. elevated storage tank.

A float switch on the sedimentation tank automatically starts and stops the raw-water pump, flocculators, four chemical feeders, two solenoid valves on the water lines to the dissolving chambers, and two electric mixers.

It is necessary to provide means for the accurate feeding of both alum and fluorspar at different raw-water pumping rates and under a wide variety of conditions of raw-water quality. The alum concentrations required may vary from 10 to more than 100 p.p.m., depending

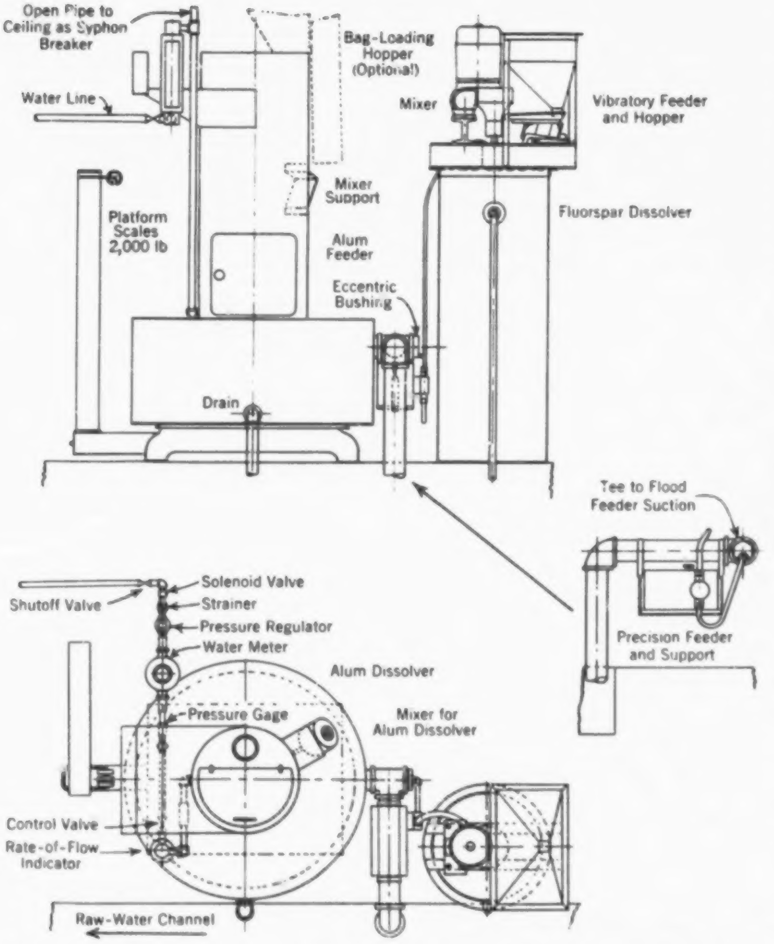
Open Pipe to Ceiling as Syphon Breaker
Water Line
Bag-Loading Hopper (Optional)
Mixer
Vibratory Feeder and Hopper
Platform Scales 2,000 lb
Mixer Support
Alum Feeder
Fluorspar Dissolver
Eccentric Bushing
Drain
Tee to Flood Feeder Suction
Precision Feeder and Support
Solenoid Valve
Shutoff Valve
Strainer
Pressure Regulator
Water Meter
Alum Dissolver
Pressure Gage
Mixer for Alum Dissolver
Control Valve
Rate-of-Flow Indicator
Raw-Water Channel

FIGURE 2.—*Schematic drawing of Fluorspar dissolver installation. This unit, designed for larger plants, is now in use at the Rosiclare plant.*

TABLE 1.—*Fluorspar feeding system at Rosiclare plant**

Desired Fluoride Addition *ppm*	Fluoride Feed Rate *gr/min*	Fluorspar Solution *per cent*	Alum Fed *ppm*	Alum Feed Rate *lb/hr*	Alum Solution		
					Solution Strength *per cent*	Feed Rate	
						lb/hr	*gpm*
0.3	0.567	0.075	25	6.25	0.75	835	1.65
1.0	1.890	0.252	25	6.25	2.52	248	0.50
1.1	2.070	0.276	25	6.25	2.76	226	0.45
0.3	0.567	0.075	65	16.20	0.75	2,160	4.30
1.0	1.890	0.252	65	16.20	2.52	645	1.30
1.2	2,260	0.302	65	16.20	3.02	537	1.10
0.3	0.567	0.075	100	25.00	0.75	3,330	6.60
1.0	1.890	0.252	100	25.00	2.52	990	2.00
1.3	2.450	0.328	100	25.00	3.28	763	1.50

* Raw-water rate, 500 gpm; dissolver upflow rate, 750 ml/min.

on the condition of the river. The fluoride content may vary from almost zero to more than 2.0 p.p.m., depending on nearby discharge of waste water from fluorspar-processing plants. In addition, it was known that, as the alum concentration increases, the fluoride loss from the treated water increases. At Bel Air, for instance, almost one-third of the added fluorides are lost when the alum concentration is 100 p.p.m. This effect is not the same for all waters or under all conditions for the same sources of water. Different types of turbidity have different effects on fluoride removal when the water is coagulated with alum.

Examples of how some of these variables are used to calculate the alum solution concentration necessary to obtain 1.0 p.p.m. fluoride in the treated water are shown in Table 1. From these examples, the strength of the alum solution can be obtained for any desired alum concentration or fluoride addition. A nomogram for the use of the water plant operators to obtain approximately this alum solution strength is given in figure 3. With the nomogram one may obtain the correct water flow rate into the alum dissolver to obtain various alum and fluoride concentrations.

So far the dissolver has produced fluoride concentrations in the predicted ratio of 10,000 p.p.m. fluorides for each 10 percent level of alum concentration. With the apparatus described the water at Rosiclare has been fluoridated to a level of 1.0 p.p.m. since Oct. 22, 1958.

The automatic removal of calcium sulfate depends on the upflow rate of the alum solution and the level of the solids below the discharge weir in the fluorspar dissolver. This water plant is operated three times each day for various periods of time depending on the rate of water consumption. At a rate of 650 ml/min, the solids rise a maximum of 10 in. requires at least 2 hours. If none of the three operating periods in any one day lasts as long as 2 hours, then no solids are discharged, provided that the initial solids level was 10 in. or more below the weir. If no solids are discharged for several days, they accumulate until the level is less than 10 in. and then begin to be discharged in less than 2 hours. The rate of rise of the alum solution must therefore be selected according to the average duration of the various operating periods. If the rate is too low, the solids accumulation becomes too deep and clogs the mixer and the lower outlet of the cone, preventing alum solution from entering the dissolver. If, on the other hand, the rate is too high, an excessive amount of fluorspar is carried up along with the calcium sulfate and lost. Ideally, that upflow rate should be used which will remove each day the same amount of calcium sulfate as was

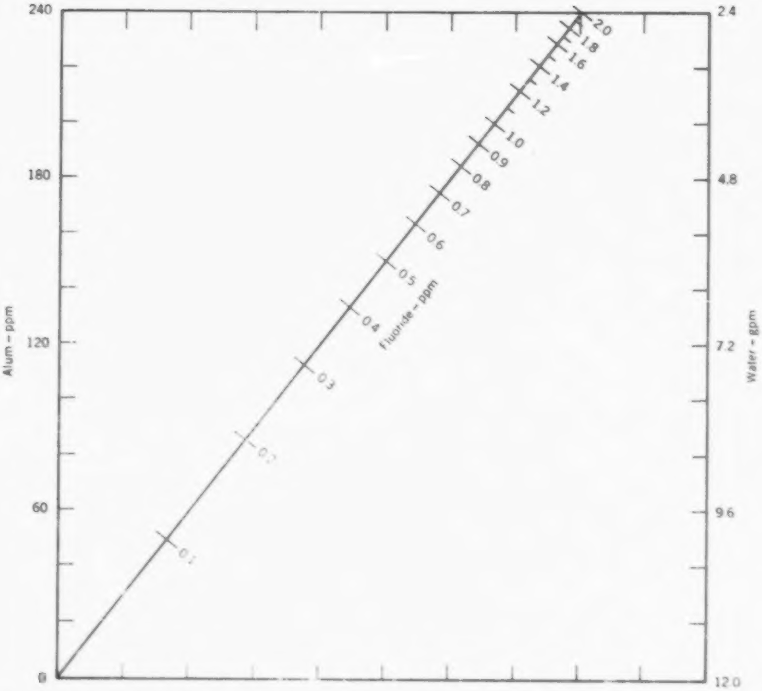

Figure 3.—*Nomogram for determining water flow rates. To use the nomogram, find the alum concentrations on the left-hand scale and lay a straightedge through it and the desired fluoride concentration on the diagonal scale. The correct water flow rate to the dissolver may then be read where the straightedge crosses the right-hand scale. The nomogram is designed for an upflow rate of 750 ml/min.*

formed, without carrying over any undissolved fluorspar. At present an upflow rate of 825 ml/min is used whenever two or more operating periods per day are prolonged for as much as 110 minutes. Under these conditions, the sludge blanket rises 16 in. in about 85 minutes to provide an average period of sludge discharge of 25 minutes. At this upflow rate, and with a 25 percent alum solution, the dissolver is capable of fluoridating up to 8.0 mgd at 1.0 p.p.m.

Conclusion

It is believed that the apparatus described can be scaled upward to accommodate the largest water treatment plants in the country. Such necessary modifications as a tank of larger diameter and a means for proportioning and introducing fluorspar could be incorporated without much difficulty.

In the United States 72 million persons are served by water utilities using coagulants in their treatment plants. This represents almost three-fourths of the population served by public water supplies. About 60 million of these people live in 325 communities whose populations are greater than 25,000. If it is assumed that at least 1 pound of fluorspar is required to fluoridate the water consumed by one person in a year, then there is a potential additional use of more than 70 million pounds of fluorspar per year for fluoridation.

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EXPERIENCE IN MAINTAINING CONSTANT FLUORIDE CONCENTRATIONS

Grace C. Scholz Spitz, Floyd B. Taylor and W. L. Harris

It is well established that optimal concentrations of fluoride in the drinking water will result in 65 percent less dental caries experience when used from birth than would result from the use of fluoride-free water (*1, 2, 3*). This knowledge has led to the widespread fluoridation of community water supplies. At present, some 32 million people in the United States use drinking water to which prescribed amounts of the fluoride ion have been added and 3 million more use water in which fluoride is naturally present in the optimal amount. It is also recognized that fluoride, when present in less than optimal amounts, at best provides less preventive benefit with regard to dental caries than it should (*4*).

There is no doubt that fluoridation can be considered one of the major preventive health measures developed in the past few decades. The responsibilty for bringing about wide-scale public acceptance of the fluoridation procedure rests with dentists, engineers, and other specialists whose role it is to effect and influence action in state and local health departments. That the responsibility in large measure has been accepted is evidenced by the extent to which fluoridation has been adopted. An impressive number of communities have instituted the procedure and a sizeable segment of the population is served fluoridated water; these are misleading measures of progress, however, if the procedure in some communities fails to provide fluoride in the proper amounts.

State health departments prescribe the level or range of levels of fluoride content to be maintained in community water supplies and, in many states, specifiy also the maximum permissible content that must not in any instance be exceeded. They provide for the chemical analysis of community water supplies, sometimes at frequent intervals in communities just beginning fluoridation, and on a regular basis at all times for the purpose of safeguarding the public health.

From the public health dentist's point of view, it is obviously equally important that the level of the fluoride content not decline below the point at which maximum benefits will be derived. This paper discusses the actual experience of three communities in respect to the precision with which they have maintained fluoride content levels as related to their objectives.

THE WATER SUPPLY SYSTEMS

The water supply system of Grand Rapids, Mich., the first community to be discussed, serves a population of about 200,000. The water plant is divided into two operating divisions requiring a fluoride feeder for each half. Gravimetric-type dry feeders are used. The plant operates as a conventional rapid sand unit, with Lake Michigan as the raw water source. Sodium fluoride is added to the water as it passes from settling basins to filters. There are no measuring devices to indicate the flow of water from the settling basins, and as a consequence the operator determines his fluoride dosage on the basis of raw water venturi readings. This arrangement requires that settling basin levels be maintained at a fairly constant figure.

City A, a town in an eastern state and the water supplier for 35,000 people, is the second community to be considered. This city, which no longer is fluoridating, derives its supply from a surface source and from several wells, each of which was equipped with its own fluoridator. At the major source, an impoundment of a small stream, sodium fluoride was added by a volumetric dry feeder in a gravity system of application. The other sources of supply are isolated wells, one of which had the same type of feeder with an auxiliary centrifugal pump to force the fluoride solution into the discharge line of the pump. At the other wells, solution-type feeders applied sodium fluoride solution to pump discharge lines.

City B, the third community for which findings are presented, is also located in the East. Its water supply system serves 12,000 people. It obtains its water from a series of wells which discharge into an open circular concrete basin. During times of peak demand, there is discharged into the reservoir an additional well-water line from a private water purveyor. A large suction line conveys water from the reservoir to a pair of centrifugal pumps. Saturated sodium fluoride solution is fed by means of a diaphragm-type pump into the suction line.

METHOD

Water samples were collected at frequent intervals and from several points in the water plants and distribution systems of the three cities under discussion for the purpose of verifying the accuracy of the procedures employed. Chemical analyses of water samples established the fluoride content.

Analysis of water samples was made: in Grand Rapids, by the water department chemist, verified in part by the Michigan State Department of Health; in City A, by the water department chemist-bacteriologist; in City B, by a chemist consultant to the water purveyor. In each instance, a Standard Methods colorimetric test was used. It may be assumed that errors in the readings of the various investigators will be of the same order of magnitude and that they are not sufficiently sizeable to introduce bias into the findings.

The resulting data were collected as part of normal record-keeping during the course of operation of the water plants. This presentation takes advantage of their availability. Approval of their use for this purpose has been obtained.

The findings were arranged in a series of frequency distributions; means and standard deviations were computed for each. These data were used to measure how accurately each system achieved its stated objective of maintaining a fluoride concentration of about 1 p.p.m.

RESULTS

The amount of fluoride applied in the Grand Rapids water plant is contingent on the amount of natural fluoride present in Lake Michigan, its source of raw water. The objective is to maintain an average between 1.0 and 1.1 parts of fluoride in one million parts of water by adding 1.0 p.p.m. fluoride to the lake water, supplementing the natural fluoride content of the lake, known to range generally from 0.04 to 0.08 p.p.m. in fluoride content.

Table 1 and figure 1 show the frequency distribution, the mean, and standard deviation of 2,796 clear-well samples read in Grand Rapids during the years 1945-1954. The mean fluoride content of these samples for this 10-year period was 1.07 p.p.m.—well within the range of the objective 1.0 to 1.1 p.p.m. The standard deviation was 0.06 p.p.m.,

TABLE 1.—*Fluoride concentrations determined from analysis of clear-well water samples collected in the Grand Rapids, Mich., water plant, 1945–1954*

Fluoride Ion Content (in ppm)	1945–54 Samples No.	1945–54 Samples Percent	1953–54 Samples No.	1953–54 Samples Percent	1951–52 Samples No.	1951–52 Samples Percent	1949–50 Samples No.	1949–50 Samples Percent	1947–48 Samples No.	1947–48 Samples Percent	1945–46 Samples No.	1945–46 Samples Percent
0.6	1	.0	0	—	0	—	0	—	0	—	1	.1
0.7	1	.0	1	.2	0	—	0	—	0	—	0	—
0.8	4	.1	0	—	0	—	0	—	1	.2	3	.5
0.9	115	4.2	6	1.2	9	1.8	10	2.0	23	3.9	67	9.5
1.0	1,947	69.8	246	48.6	304	60.1	362	72.7	497	85.4	538	76.4
1.1	671	24.0	251	49.6	182	36.0	98	19.7	55	9.4	85	12.1
1.2	51	1.8	2	.4	11	2.1	26	5.2	4	.7	8	1.2
1.3	2	.1	0	—	0	—	1	.2	0	—	1	.1
1.4	0	—	0	—	0	—	0	—	0	—	0	—
1.5	0	—	0	—	0	—	0	—	0	—	0	—
1.6	0	—	0	—	0	—	0	—	0	—	0	—
1.7	1	.0	0	—	0	—	0	—	0	—	1	.1
1.8	0	—	0	—	0	—	0	—	0	—	0	—
1.9	1	.0	0	—	0	—	1	.2	0	—	0	—
2.0	0	—	0	—	0	—	0	—	0	—	0	—
2.1	1	.0	0	—	0	—	0	—	1	.2	0	—
2.2	0	—	0	—	0	—	0	—	0	—	0	—
2.3	0	—	0	—	0	—	0	—	0	—	0	—
2.4	1	.0	0	—	0	—	0	—	1	.2	0	—
Total	2,796	100.0	506	100.0	506	100.0	498	100.0	582	100.0	704	100.0
Mean		1.07		1.09		1.08		1.08		1.06		1.05
Standard deviation		.06		.05		.10		.07		.08		.06

indicating that 95 percent of the readings ranged from 0.95 to 1.19 p.p.m. fluoride and showing remarkable precision in achieving the desired fluoride content.

To demonstrate that deviations from the mean during this 10-year period are comparable for shorter time intervals, table 1 and figure 1 also list frequency distributions, means, and standard deviations of the clear-well samples for the five two-year periods between 1945 and 1954. During each of these biennial periods, the mean of the readings was close to the mean for the entire period and the standard deviation was comparable to that of the summary findings for the total group. The five means ranged from 1.05 to 1.09 p.p.m., with standard deviations of 0.04 to 0.10.

The objective was to add 1.0 p.p.m. fluoride to the water supply. Only 29 of the 346 samples contained as much as 1.0 p.p.m. fluoride. The mean amount of fluoride, 0.62 p.p.m., found in the samples falls far short of the amount required to provide optimum benefits. The size of the standard deviation, 0.23 p.p.m., points up the wide variation in the readings.

Readings of 316 samples from the four water supply sources in City A collected during the same time period gave a mean of 0.59 p.p.m fluoride and a standard deviation of 0.36 — which closely parallels the fluoride content found in the distribution system.

City A has ceased to fluoridate by action of the governing council following suspension of the procedure by the water superintendent.

Table 2.—*Fluoride concentrations determined from analysis of water samples collected from 15 distribution-system taps in city A, January, 1950 to April, 1952*

Fluoride Ion Content (in ppm)	Samples	
	Number	Per cent
0.0	6	1.7
0.1	1	.3
0.2	6	1.7
0.3	18	5.2
0.4	35	10.1
0.5	74	21.4
0.6	61	17.6
0.7	55	15.9
0.8	35	10.1
0.9	26	7.5
1.0	22	6.4
1.1	3	.9
1.2	1	.3
1.3	0	—
1.4	3	.9
Total	346	100.0
Mean	0.62	
Standard deviation	0.23	

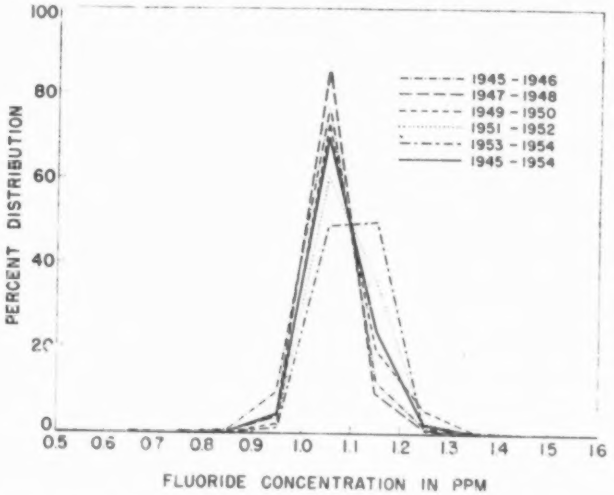

Figure 1.—*2,796 water samples from clear well in Grand Rapids, Mich.*

In the course of this same 10-year period 5,386 water samples were drawn for varying lengths of time at 11 points in the Grand Rapids distribution system. The mean readings for fluoride content of water collected at these specified sites ranged from a low of 1.04 p.p.m. to a high of 1.12 p.p.m., with standard deviations that ranged from 0.03 to 0.10, making these findings quite comparable with those obtained from clear-well readings.

It is apparent that fluoride-content determinations in the water plant yielded data quite comparable to those obtained from the analysis of distribution system findings during the same time period.

Table 2 and figure 2 present findings for 346 samples collected during the period January, 1950–April, 1952, from 15 distribution-system taps in City A.

Table 3a.—*Fluoride concentrations determined from analysis of water samples collected from the distribution system in city B, November, 1950 to May, 1952*

Fluoride Ion Content (in ppm)	Samples	
	Number	Per cent
0.0	28	17.0
0.1	1	.6
0.2	11	6.7
0.3	0	—
0.4	9	5.5
0.5	1	.6
0.6	21	12.7
0.7	9	5.5
0.8	22	13.3
0.9	32	19.4
1.0	12	7.3
1.1	7	4.2
1.2	4	2.4
1.3	0	—
1.4	1	.6
1.5	4	2.4
1.6	1	.6
1.6+	2	1.2
Total	165	100.0
Mean	0.66	
Standard deviation	0.41	

Table 3a and figure 3 present frequency distribution, mean, and standard deviation of 165 water samples collected in the distribution system of City B during the period between November, 1950, and May, 1952. Although the objective was to provide 1.0 p.p.m. of fluoride, the mean of these samples was 0.66 p.p.m. and the standard deviation 0.41, indicating a wide variation in the readings.

In verification of these findings, data collected at the pumping station during a portion of the same period (86 readings) show a mean of 0.76 p.p.m. and a standard deviation of 0.32.

The officials of City B, aware that the resultant amount of fluoride provided in the system was inadequate, introduced corrective measures. The results are shown in table 3b and figure 3, which give the frequency distribution, mean, and standard deviation of 130 distribution-system water samples drawn during the subsequent period of May–October, 1952. The mean of these samples, 0.88 p.p.m., is considerably closer to the objective of 1.0 p.p.m. than that

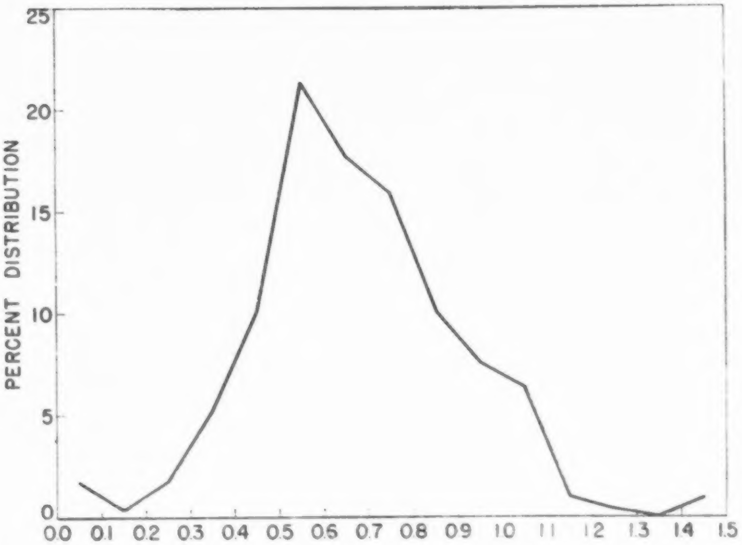

Figure 2.—*Water samples from 15 distribution-system taps in city A, January, 1950–April, 1952.*

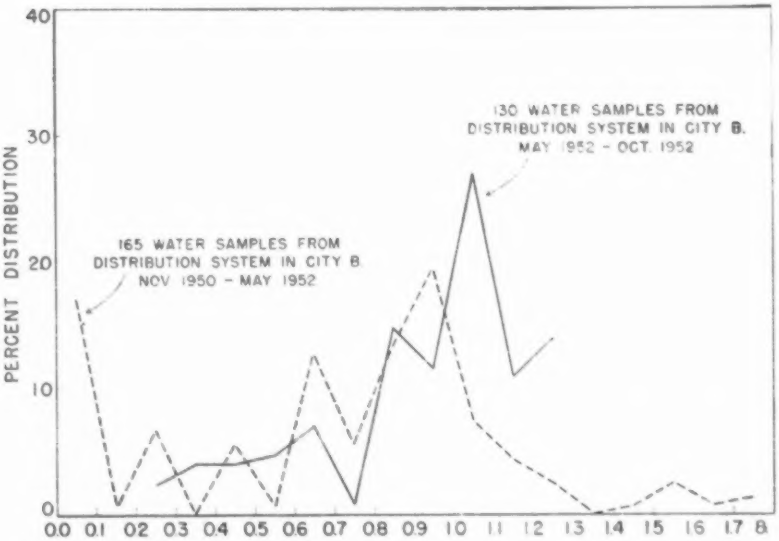

Figure 3.—*Water samples from the distribution system in city B, specified periods.*

which was obtained earlier. The standard deviation of the mean, 0.26 p.p.m., is still quite large but demonstrates a greater tendency of the daily readings to cluster than those collected in the preceding time interval.

While fluoridation in City B had not quite achieved the level at which optimum protection against tooth decay would be afforded, there had been a marked improvement in procedure.

For ready comparison, figure 4 gives the frequency distributions of the samples drawn in Grand Rapids, in City A, and in City B both before and after the adoption of corrective measures.

Table 3B.—*Fluoride concentrations determined from analysis of water samples collected from the distribution system in city B, May to October, 1952*

Fluoride Ion Content (in ppm)	Samples Number	Per cent
0.0	0	—
0.1	0	—
0.2	3	2.3
0.3	5	3.9
0.4	5	3.9
0.5	6	4.6
0.6	9	6.9
0.7	1	.8
0.8	19	14.6
0.9	15	11.5
1.0	35	26.9
1.1	14	10.8
1.2	18	13.8
Total	130	100.0
Mean	0.88	
Standard deviation	0.26	

DISCUSSION

Three water supply systems have been discussed, in each of which a somewhat different procedure for application of the fluoridation process is used. While any given procedure may present unique engineering difficulties, one problem which these three have in common, and with which the operators of each of the 800 water supply systems now fluoridating are confronted, is the consistent maintenance of a selected fluoride level. Earlier studies have demonstrated that water-borne fluorides, present in optimal amounts and used from birth, are an effective means by which dental caries can be reduced. The extent to which protection is afforded, however, is entirely dependent on the maintenance of a constant level of optimal fluoride content.

The fluoridation procedure presents no unusual technical difficulties but it does require that the water plant operator demonstrate the same careful practice he is accustomed to exercise in the application of any water treatment process. Accurate fluoride feeding may in some instances be a difficult task but it is never an impossible one.

Ten years' experience in Grand Rapids and in Newburgh, N.Y., (*5*) have demonstrated the practicability of maintaining constant, optimal fluoride levels in the controlled fluoridation procedure. The experience in City B shows clearly, too, that improvement is possible when the deficiency is recognized and correction is sought.

Unfortunately City A and City B are

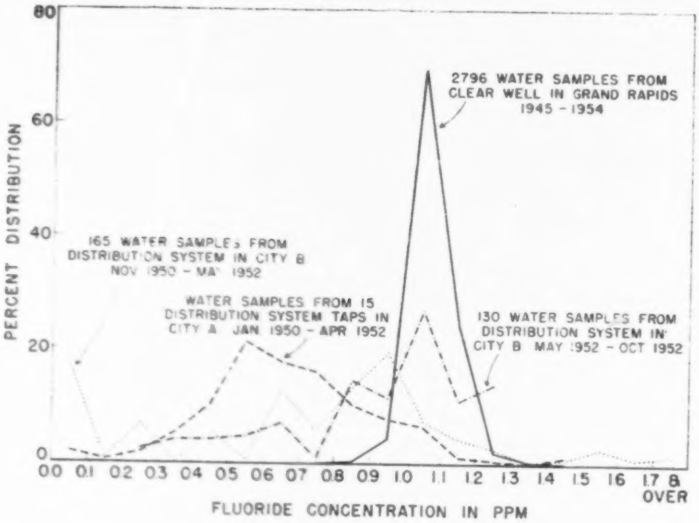

FIGURE 4.—*Water samples from distribution systems in three cities, specified periods.*

not unique as communities in which fluoridation has been functioning on a less than adequate basis. It may be that some benefit is derived from fluoridation even when the procedure is inadequately maintained; if there is, however, it certainly falls far short of what might be achieved. Dental examinations will reveal some years after the start of fluoridation whether reductions in dental caries prevalence are less than they should have been. (*6*) Periodic current determinations of fluoride content and immediate correction of observed deficiencies can prevent this eventuality. A casual review of fluoride test data is insufficient basis upon which to judge the true trend of the results of the fluoridation procedure. An analysis, by simple statistical methods, of series of test results, preferably at intervals not exceeding three months, is needed to determine the true picture.

State health departments frequently establish a maximum fluoride concentration never to be exceeded. Perhaps it is too readily assumed that the job has been satisfactorily done if this condition is met in any given analysis of a water sample. Recommended levels of fluoride concentration are also established in many states, but no great concern is evidenced when fluoride concentrations are found below the lowest recommended level. Just as it is clearly the obligation of those concerned with water fluoridation to see that the levels do not exceed maximal standards, so it is their obligation to maintain optimal standards.

The health department engineer bears the major direct responsibility for surveillance of the controlled fluoridation process. He is closest to the waterworks operator and usually has the latter's confidence. The engineer is therefore in an excellent position to follow the trend of fluoridation from his own and the operators' reports. It is his duty to note serious deviations from proper practice and to make recommendations for corrective action.

A less direct responsibility lies with the dentist. Unfortunately, however, from the point of view of the public—the user of fluoridated water—it is the dentist who has predicted and promised the benefits that can be derived from fluoridation. It is he who has assured them that children's teeth will be better preserved if they drink fluoridated water. The dentist's subsequent indifference to the necessity for maintaining a precise fluoride level and his failure to exercise the necessary vigilance through proper engineering channels can only bring him discredit should fluoridation be condemned because inadequate procedure has produced inadequate results.

The situation is only made worse because the results are not immediately measurable. The time lag between application of the procedure and determination of its effect means that the damage is done before it can be rectified. It will be too late to prevent the inevitable recriminations five or ten years from now should it become evident then, by measurement of dental caries prevalence levels, that adequate fluoride concentrations have not been maintained.

In the last analysis, fluoridation as a public health measure will not be judged by the maximum results that a properly conducted study procedure can effect. It will be judged by its actual achievement in preventing dental caries in all communities of all states where it is practiced. Can we be assured that the predictions of the dentists of today will stand the time-lag test? Today's predictions will meet future public scrutiny only if the engineers and the dentists of today suit the action to the word and make certain that what is so eagerly preached is also meticulously practiced.

SUMMARY

1. Water samples collected in three cities for varying time intervals have been analyzed to determine constancy and adequacy of the fluoride levels that were maintained.

2. Constancy of fluoride readings at the optimal level with a small standard deviation has been demonstrated in one city, Grand Rapids, Mich.

3. Average fluoride content in City A fell far short of the objective and demonstrated a large standard deviation in the daily readings during the time period for which data were collected.

4. Average fluoride content in City B fell short of the objective during the initial test period and showed wide variation during this interval. During a succeeding test period in which efforts for improved controls were attempted, the average fluoride content more nearly approximated the objective and individual readings demonstrated much less variability.

5. It is apparent that maximal benefits in dental caries prevention will not be achieved unless optimal fluoride concentration is maintained. Mindful awareness of any failure to maintain this objective should prompt immediate correction of a deficient application of the technic. Delay until it is evident that anticipated reductions in dental caries prevalence levels have not been achieved can only bring underserved discredit to one of the principal public health measures of this era.

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CUMULATIVE DISTRIBUTION METHOD FOR EVALUATING SERIES OF FLUORIDE ION TEST RESULTS

Floyd B. Taylor

Spitz, Taylor, and Harris (1) showed that results of fluoride ion tests on samples taken from public water supply distribution systems could vary widely and fall significantly below optimum values for the area concerned. They predicted also that maximum benefits in dental caries prevention would not be achieved unless optimum fluoride concentrations were consistently maintained. That this was a true assumption was demonstrated by Chrietzberg and Lewis (2) in their study of 8,272 school children in De Kalb County, Ga.

Visual inspection of a series of fluoride ion test results does not always reveal the true course of the fluoridation process. For example, in reviewing a column of many figures, the small values may appear to be insignificant when seen together with a greater number of larger values. After systematic arrangement and analysis of the total data, however, it is sometimes found that the small values have depressed the mean to a point where corrective action is imperative. A systematic analysis of a series of fluoride ion test results at regular intervals is also recommended to detect any trend of mean values that may be significant.

In an analysis of fluoride ion test results from a public water supply (3) the statistical method of cumulative distribution was found to be a simple and useful tool. As this method is not generally known to water utility operators and public health engineers, a demonstration of the method, as it can be applied to fluoridation, may be helpful. The fluoride ion test data are from actual situations.

DEFINITIONS

The statistical terms used in this article are defined as follows:

Cumulative distribution: the successive total number of samples with a fluoride ion concentration up to and including a given value in the series. It is expressed as a percentage.

Median: the fluoride ion value so chosen in a series that half the sample test results are greater than, and half less than, this value.

Mean: the arithmetic average fluoride ion value of a series.

Range: the difference in fluoride ion concentration between maximum and minimum values in a series.

Standard deviation: a measure of variation about a mean. When considered together with the mean, it is a central range containing about two-thirds the test results in the series—that is, the mean fluoride ion concentration plus and minus the standard deviation is the range within which about two-thirds of the test results of the series fall.

PROCEDURE

1. Gather a series of test results over a period of a few weeks; a period not exceeding 3 months is recommended. The raw data may be similar to the following (values represent fluoride ion content, in parts per million, in distribution system samples):

0.2	1.0	1.2	1.0	1.0	1.0	1.0	0.6	1.2	0.6
0.8	1.0	1.2	0.8	1.0	1.0	0.2	0.7	1.2	0.4
0.3	0.2	1.0	0.3	0.8	1.2	1.0	1.0	0.3	0.5
0.8	1.0	1.1	0.6	0.8	0.4	0.8	1.2	0.6	0.6
0.3	1.0	1.0	0.5	0.9	0.9	0.8	1.0	0.8	0.3
0.4	0.5	1.1	0.6	0.9	0.9	0.8	1.1	1.2	0.6
0.5	1.0	1.0	0.6	1.0	1.1	0.8	1.1	0.8	1.2
0.9	1.0	1.1	0.8	1.2	1.1	0.8	1.1	0.8	0.8
1.0	1.1	1.0	0.9	1.0	0.4	0.9	1.1	1.2	0.8
0.6	1.0	1.0	0.9	1.0	0.5	0.9	1.1	1.2	0.8
0.8	1.0	1.1	0.9	1.2	1.0	0.9	1.0	0.4	0.9
0.9	1.1	1.2	1.0	1.0	1.0	1.2	1.2	0.5	0.9
1.0	1.0	1.1	1.2	1.0	1.0	1.2	1.2	0.9	0.8

2. Construct a table with columns and headings similar to those in Table 1.

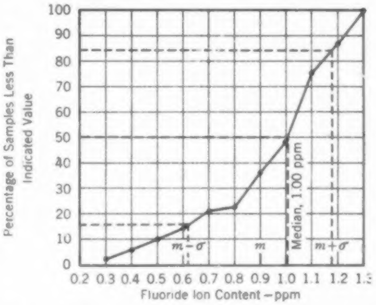

FIGURE 1.—*Cumulative distribution of 130 fluoride ion test results. The letter* m *represents the mean, 0.9 ppm;* σ *represents the standard deviation of 0.28 ppm.*

TABLE 1.—*Suggested arrangement of data —130 fluoride ion test results.**

(Column 1) Fluoride Ion Content *ppm*	(Column 2) No. of Samples	(Column 3) Cumulative No. of Samples	(Column 4) Cumulative No. of Samples %
0.2	3	3	2.3
0.3	5	8	6.2
0.4	5	13	10.0
0.5	6	19	14.6
0.6	9	28	21.6
0.7	1	29	22.3
0.8	19	48	36.9
0.9	15	63	48.5
1.0	35	98	75.5
1.1	14	112	86.2
1.2	18	130	100.0

* The identification of each column, shown in parentheses, is for purposes of discussion.

3. Determine the range of test values covered by the raw data. In Column 1 of the table, cover the range in equal increments. Increments of 0.1 ppm are

recommended when the data have one significant decimal place; 0.05 ppm, when two significant decimal places are found.

4. In Column 2 of the table, enter opposite each value in Column 1 the number of samples that tested at the value. For example, in the raw data, it is found that three samples tested at 0.2 ppm, five at 0.3 ppm, and so on.

5. In Column 3 of the table, cumulatively add the figures in Column 2. The last entry must equal the total number of samples, and thus provides a check on the arithmetic.

6. In Column 4, enter opposite each number in Column 3 the percentage that number is of the total number of samples. Thus, 3 samples are 2.3 per cent of 130, 29 samples are 22.3 per cent, and so on. The last figure must be 100 per cent.

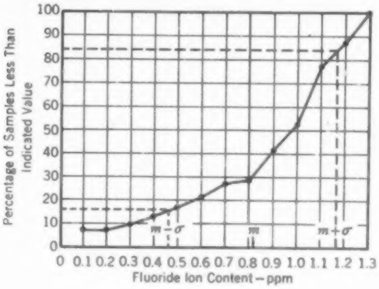

Figure 2.—*Cumulative distribution of 141 fluoride ion test results. The letter* m *represents the mean, 0.815 ppm;* σ *represents the standard deviation of 0.35 ppm.*

7. On a sheet of arithmetic graph paper, construct, to a convenient scale, two axes. Call the vertical scale percentage of samples less than the indicated value; call the horizontal scale fluoride ion content in parts per million. The horizontal scale must cover the range and one step above the upper limit of the range (Fig. 1).

8. Plot the data in Column 4 of the table against the data in Column 1, and connect the points by straight lines. Plot the points one increment above the value at which the percentage is shown. For example, plot 2.3 per cent opposite 0.3 ppm, for actually 2.3 per cent of the samples are less than 0.3 ppm in fluoride ion content.

9. From the 16 per cent and 84 per cent points on the vertical scale, intersect the plotted curve with horizontal dotted lines, and project them downward vertically to intersect the horizontal scale. On this scale, bisect the distance between the two points and determine the value of the new point. This is a reasonable approximation of the mean value of the series. In the example given (Fig. 1), the mean value is 0.9 ppm.

10. Determine the value, in parts per million from the horizontal scale, of the distance from the mean point to either of the points from which it was found. This value—0.28 ppm, in the example cited—is a reasonable approximation of the standard deviation of the series.

If it is desired to find the median of the series, draw a horizontal dotted line from the 50 per cent point on the vertical scale to the plotted line; then draw a vertical dotted line to the horizontal scale. The point at which the vertical line intersects the horizontal scale is the median value of the series.

INTERPRETATION OF RESULTS

Arroe (*4*), Spitz (*1*), and Taylor (*3*) showed that it is possible to maintain fluoride ion values so that the mean of a series of test results varies very little from the desired optimum value, and so that the test results cluster closely about the mean value. When this occurs, the standard deviation is found to be only a few thousandths of a part per million. More important, when the means of series of test results become and remain little or no different from the optimum value, and when the standard deviation becomes and remains small, optimum caries reduction may be anticipated.

Table 2.—*Suggested arrangement of data —141 fluoride ion test results*

Fluoride Ion Content *ppm*	No. of Samples	Cumulative No. of Samples	Cumulative No. of Samples %
0.0	11	11	7.81
0.1	0	11	7.81
0.2	3	14	9.94
0.3	5	19	13.49
0.4	5	24	17.04
0.5	6	30	21.30
0.6	9	39	27.69
0.7	1	40	28.40
0.8	19	59	41.89
0.9	15	74	52.54
1.0	35	109	77.39
1.1	14	123	87.33
1.2	18	141	100.00

In the locality from which the series of test results cited above was gathered, the optimum fluoride ion value and the value that the water plant operators sought to maintain were both 1.0 ppm. It is seen (Fig. 1) that the mean value of the series examined was 0.9 ppm—0.1 ppm less than the optimum—and, more important, the large standard deviation of 0.28 ppm showed a wide scattering of results. If these conditions prevailed over an extended period, there would, undoubtedly, be less of a reduction of caries in the area than should be expected. This may be further understood by comparing the above data first with a series that was less satisfactory, and then with one that showed an excellent fluoridation experience.

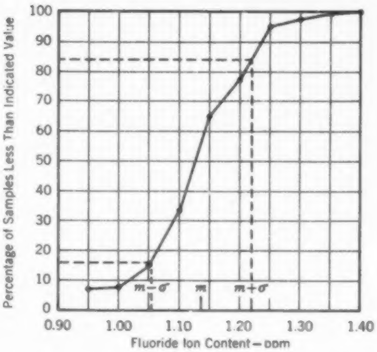

Figure 3.—*Cumulative distribution of 432 fluoride ion test results. The letter* M *represents the mean, 1.135 ppm;* σ *represents the standard deviation of 0.083 ppm.*

Table 2 and Figure 2 depict a series in which, by the above method, the mean was found to be 0.815 ppm and the standard deviation 0.35 ppm. The optimum value sought to be maintained was 1.0 ppm.

In contrast to both the above series are the results of analyzing 432 test results from another supply. The optimum value for the area was 1.1 ppm. The cumulative distribution analysis (Table 3 and Fig. 3) showed a mean value of 1.135 ppm and a standard deviation of only 0.083 ppm.

Table 3.—*Suggested arrangement of data —432 fluoride ion test results*

Fluoride Ion Content *ppm*	No. of Samples	Cumulative No. of Samples	Cumulative No. of Samples %
0.90	27	27	6.25
0.95	4	31	7.18
1.00	35	66	15.28
1.05	79	145	33.56
1.10	138	283	65.51
1.15	52	335	77.55
1.20	79	414	95.83
1.25	9	423	97.92
1.30	8	431	99.77
1.35	1	432	100.00

SUMMARY

The cumulative distribution method for analyzing series of fluoride ion test results has been found to be a simple and useful method for determining the true course of the fluoridation process. Optimum results of fluoridation, in terms of caries reductions, can be anticipated only when mean fluoride ion values are consistently maintained at the optimum value for the area concerned. A low standard deviation indicates a compactness of the daily test result values about the mean, which should lead to a more satisfactory fluoridation experience.

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Caries Inhibition by Fluoride Not Provided by Community Drinking Water

The quantity of fluoride ingested by children aged 1–12 years in drinking water containing 1.00 p.p.m. of fluoride, under average conditions is approximately 0.5 mg. to 1.0 mg. fluoride daily. When ingested daily during the first 12 years of life this quantity of fluoride, additional to 0.2–0.5 mg. in the daily food, has a significant caries inhibitory effect.

A daily supplement of fluoride provided by a tablet containing 1.0 mg. of fluoride as sodium fluoride is an effective means of caries prevention. This use of fluoride compares favorably with fluoridated drinking water in its preventive effect on dental caries.

PUBLICATIONS

McClure, F. J.: Ingestion of Fluoride and Dental Caries—Quantitative Relations Based on Food and Water Requirements of Children 1 to 12 Years Old. Am. J. Diseases of Children *66*: 362–369, 1943.

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INGESTION OF FLUORIDE AND DENTAL CARIES. Quantitative Relations Based on Food and Water Requirements of Children 1 to 12 Years Old

F. J. McClure

With few exceptions the biochemistry of fluorine emphasizes its toxic features. The production of endemic dental fluorosis (mottled enamel) in human beings by fluorine in drinking water (*1*) is an outstanding example of the toxic effect of an excessive intake of the element. The recent discovery of the inhibitory effect of trace quantities of fluorine in drinking water on dental caries in children aged 12 to 14 years (*2*) and in young adults (*3*) marks the first indication that fluorine may have a beneficial role in animal physiology. This inhibitory effect of fluorine on dental caries is shown by data in table 1 and in figure 1, taken from a recent report by Dean, Arnold and Elvove (*2b*). The figures in table 2 are from a report by Deatherage (*3*) based on results of dental examinations of selectees of the current Selective Service law. The effect of fluorine present in drinking water is most striking, the total incidence of dental caries being reduced a third to one half by this water-borne element.

Prior to the discovery of the fluorine-dental caries association, any suspicion as to the physiologic importance of fluorine gained support principally from the observation that fluorine was present in trace quantities in many plant and animal tissues. Even this widespread occurrence of fluorine seemed accidental. In general, interest centered in the relation of fluorine to calcified tissues, i.e. the bones and teeth. Gautier and Clausmann (*4*) and Gautier (*5*) have speculated on the association of fluorine with the hardness and the resistance (*d'inaltérabilité chimique*) of body tissues which they found to contain the most notable quantities of fluorine. The improved performance of dental tissue (resistance to caries) due to the ingestion of fluorine may possibly agree with Gautier's hypothesis. Although the data are somewhat limited, the results of Armstrong and Brekhus (*6*) suggest that carious teeth may actually contain less fluorine than noncarious teeth.

While the observed fluorine–dental caries relation is suggestive of the effect of a trace element, it cannot be said that fluorine is an essential element of the diet in the accepted sense of the term. It has not been shown that the complete withdrawal of fluorine from otherwise adequate diets produces fatal or disabling symptoms. Dental caries cannot be classified as a fluorine deficiency disease until it has been demonstrated that inadequate amounts of fluorine in the diet invariably produce the disease. Thus far such observations have not been made. Attempts have been made to test the necessity of fluorine in the diet (*7*), the white rat being used as the experimental subject. While dietary fluorine was reduced to an extremely low level in these studies, particularly in the experiment by Sharpless and McCollum (*7a*), traces of fluorine still remained in these diets. The results of these experiments gave no evidence that the rat requires fluorine. In contrast to the human subject, however, the rat has not proved to be notably susceptible to spontaneous dental caries.

The hygienic value of fluorine as a preventive of caries suggests the inclusion of fluorine in the diet in certain optimum amounts. The dental tissues are extremely sesitive to fluorine during the formative period, i.e., during the first seven or eight years of life (with exclusion of the third molar teeth, which are not completely developed until the 12th or 14th year). The addition of fluorine to children's diets during the first seven or eight years of life offers great promise of reducing the prevalence of dental caries. At the present time the quantity of fluorine in the diet associated with caries-inhibitory effects is defined in terms of fluorine present in drinking water. As shown in table 1, such quantities are of the order of 0.5 to 1.4 parts per million or more. In consideration of the significance of the fluorine-dental caries relation and the thereby enhanced nutritional importance of fluorine, it is desirable to have some idea of the approximate quantities being ingested daily in food and certain drinking waters. The figures in table 3 are presented, therefore, to show the approximate quantities of fluorine ingested daily through the use of drinking water containing 1 part per million and in the consumption of food containing trace amounts. One part per million was selected as the concentration of fluorine

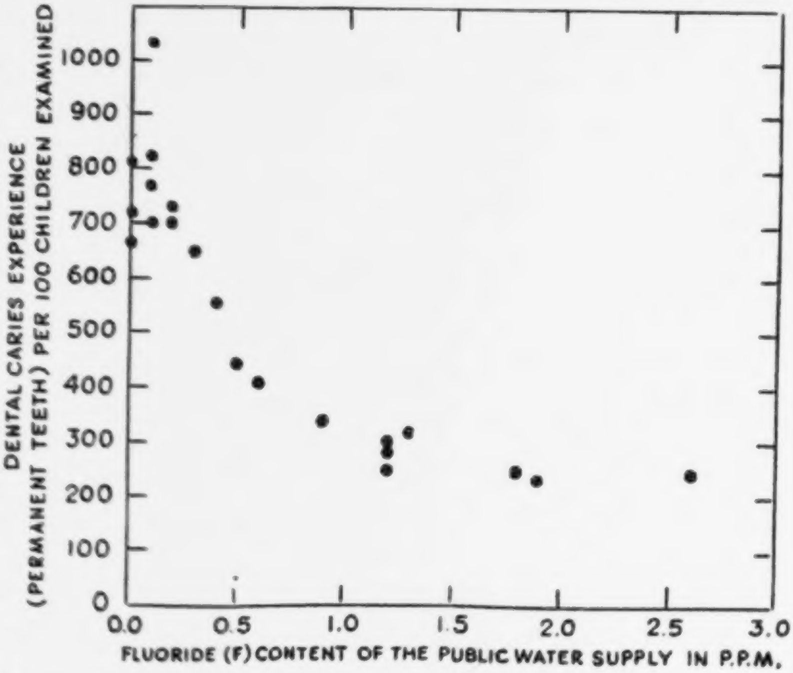

FIGURE 1.—*Relation between the incidence of dental caries in permanent teeth observed in 7,257 selected 12 to 14 year old white school children of 21 cities of 4 states and the fluoride content of the public water supply (from Dean, Arnold and Elvove 2(b)).*

in drinking water because according to both the epidemiologic evidence (*2a*) and the standards for drinking water of the Public Health Service (*8*) this is a permissible level. While final judgment regarding the possibility of other physiologic effects resulting from this quantity of fluorine (1 part per million) in drinking water is not justified, the evidence available at this time does not suggest that harm will result when this much fluorine is present in children's diets from perhaps the age of 1 year through the seventh or eighth year or even to an older age.

Drinking of water is a variable factor, especially among children, whose drinking habits are greatly influenced by muscular activity as well as by atmospheric temperature and humidity. It is likewise true of children's diets especially that the requirement of water is met largely by preformed water in the food or by liquid food, particularly milk. The water deficit made up by drinking water may be a relatively small fraction of the total daily amout or water ingested. For the estimates of ingestion of water as drinking water per se, as shown in table 3, the method of estimating the daily water requirement of the average child and adult proposed by Adolph (*9*) was adopted. Adolph estimated the daily water requirement as equal to 1 cc. per calory of energy in the daily diet. In consideration of the numerous factors influencing water-drinking habits, two estimates of the amount of water drunk have been made: (*a*) Drinking water was estimated to equal 25 percent of the daily requirement and (*b*) to equal 33 percent of this requirement. These approximations apply to children's drinking habits extending over an entire year in a temperate climate. No doubt considerable fluctuation would occur throughout the year and average quantities drunk would vary somewhat with the climate.

The figures for water consumption in table 3 may be compared with other estimates and actual measurements of water drinking. Macy (*10*) found that the total amount of water consumed daily by children 8 to 12 years of age ranged from 1,658 to 1,745 cc.; 449 to 568 cc. of these amounts, i.e. about 25 to 30 percent, was ingested as drinking water. Richter and Brailey (*11*) regarded 2,400 cc. as a good average for water intake daily by an average man weighing 65 kg. Another estimate of the average water requirement of children aged 1 to 18 years is 1,100 to 2,700 cc. daily (*12*). Magee (*13*) studied the fluid intake of persons aged 8 to 80 years. Total water consumed, i.e. raw water, including water used in cooking, averaged 1,324 cc. for males and 1,551 cc. for females daily. Additional water in milk and other foods accounted for a daily water intake of 2,081 cc. for males and 2,195 cc. for females. Rowntree (*14*) estimated the average diet (food) to contain 1,000 cc. of water daily, made up of water naturally in food, water from oxidation of food and water added during cooking. The remainder of the total daily water requirement would be supplied by water drunk per se, and by other beverages. These later quantities totaled about 1,200 to 1,600 cc. daily. In a previous publication I estimated the average daily consumption of drinking water by the average adult to equal 1,200 to 1,500 cc. (*15*). For an excellent discussion of water metabolism the article by Adolph (*9*) should be consulted.

Water added to food during cooking processes is probably more unpredictable than any of the various factors involved in estimating water ingestion. In order to account for the addition of a quantity of fluorine to food during cooking, the calculations in table 3 include estimates of (*a*) 10 percent and (*b*) 20 percent of the total food-borne water as consisting of water of drinking water origin.

The amount of fluorine ingested in food seems also to be surrounded with considerable uncertainty. Analytic methods for determination of fluorine have not progressed to the point where trace quantities, such as occur in most foods, can be determined with entire satisfaction. A list of foods and food constituents and their fluorine content as reported in the literature, is shown in table 4. Most foods appear to contain somewhat less than 1 part of fluorine per million parts of dry substance. Although fish foods and teas may contain on the average as much as 8 to 12 and 30 to 60 parts per million of fluorine respectively, these foods do not usually occur in appreciable quantities in children's diets. Because of obvious uncertainties surrounding the quantity and quality of various foods in children's diets, four levels of fluorine in foods have been selected as falling within the range apt to be present in the mixed dry substance of the average diet.

The calory allotments shown in table 3 are taken from recent standards set by the Committee on Food and Nutrition of the National Research Council (*16*). The total dry substance of the diet has been estimated on the assumption that 1 gm. of dry substance comprising the average mixed diet will have an average energy value of 4.5 calories. The total dry substance was estimated, therefore, by dividing the calory allotment by this figure. The fluorine content of the diet was based on this estimate of dry substance.

In observations on young rats, Lawrenz, Mitchell and Ruth (*17*) and

Table 1.—*Relation of fluorine concentration of public water supplies and dental caries (data from Dean, Arnold and Elvove (2b))*

Number of Children Examined	Age, Years	Total Incidence of Dental Caries (Missing Teeth, Untreated Teeth and Filled Teeth per 100 Children)	Fluorine in Public Water Supply, Parts per Million
3,867	12-14	745	<0.5
1,140	12-14	425	0.5 to 0.9
1,403	12-14	300	1.0 to 1.4
847	12-14	250	>1.4

Table 2.—*Dental caries experience of draft selectees living in endemic fluorine areas compared with selectees living in nonendemic areas (data from Deatherage (3))*

	Age, Years	Number Examined	Total Incidence of Caries (Teeth with Untreated Caries, Filled Teeth, Extraction Indicated and Missing Teeth per 100 Selectees Examined)	Area *
Entire life spent in respective areas	21-24	130	510	Endemic
	21-24	112	1,011	Nonendemic
	25-28	34	609	Endemic
	25-28	52	1,029	Nonendemic
	All ages	164	530	Endemic
	All ages	164	1,016	Nonendemic
First eight years, but not entire life spent in respective areas	21-28	77	581	Endemic
	21-28	82	1,077	Nonendemic

* Endemic areas are those where fluorine in the drinking water causes mottled enamel. Fluorine, although causing the teeth to be mottled if present in quantities above 1 part per million in drinking water, also inhibits dental caries in these larger amounts.

TABLE 3.—*Estimated daily intake of fluorine from drinking water containing 1 part per million of fluorine and from food containing 0.1 to 1 part per million of fluorine in the dry substance*

Age (years)	1 to 3	4 to 6	7 to 9	10 to 12
Energy allowance (calories)	1,200	1,600	2,000	2,500
Water requirement (cc.)	1,200	1,600	2,000	2,500
Drinking water consumption				
(1) When water drunk is equal to 25 per cent of the total daily water requirement and (*a*) 10 per cent and (*b*) 20 per cent of the total water content of the food is of drinking water origin, the total daily consumption of drinking water would equal:				
(*a*)	390 cc.	520 cc.	650 cc.	812 cc.
(*b*)	480 cc.	640 cc.	800 cc.	1,000 cc.
(2) When water drunk is equal to 33 per cent of the total daily water requirement and (*c*) 10 per cent and (*d*) 20 per cent of the total water content of the food is of drinking water origin, the total daily consumption of drinking water would equal:				
(*c*)	480 cc.	640 cc.	800 cc.	1,000 cc.
(*d*)	560 cc.	745 cc.	933 cc.	1,165 cc.
Total daily fluorine ingested from drinking water containing 1 part per million of fluorine under the preceding conditions of water ingestion would equal:				
In water intake (*a*)	0.390 mg.	0.520 mg.	0.650 mg.	0.810 mg.
In water intake (*b*) and (*c*)	0.480 mg.	0.640 mg.	0.800 mg.	1.000 mg.
In water intake (*d*)	0.560 mg.	0.745 mg.	0.930 mg.	1.165 mg.
Total dry substance in daily food allowance when 1 Gm. of dry substance of the food furnished 4.5 calories of energy				
Total daily intake of dry substance	265 Gm.	356 Gm.	445 Gm.	555 Gm.
Fluorine ingested daily in food in which the dry substance of the food contained the following concentrations of fluorine:				
(*a*) 0.10 part per million	0.027 mg.	0.036 mg.	0.045 mg.	0.056 mg.
(*b*) 0.20 part per million	0.053 mg.	0.071 mg.	0.089 mg.	0.111 mg.
(*c*) 0.50 part per million	0.133 mg.	0.178 mg.	0.223 mg.	0.278 mg.
(*d*) 1.00 part per million	0.265 mg.	0.360 mg.	0.450 mg.	0.560 mg.
Estimated total daily fluorine ingested in food and drinking water:				
Food (*a*) plus water (*a*)	0.417 mg.	0.556 mg.	0.695 mg.	0.866 mg.
Food (*b*) plus water (*a*)	0.443 mg.	0.591 mg.	0.739 mg.	0.921 mg.
Food (*c*) plus water (*a*)	0.523 mg.	0.698 mg.	0.873 mg.	1.088 mg.
Food (*d*) plus water (*a*)	0.655 mg.	0.880 mg.	1.100 mg.	1.370 mg.
Food (*a*) plus water (*b*) or (*c*)	0.507 mg.	0.676 mg.	0.845 mg.	1.056 mg.
Food (*b*) plus water (*b*) or (*c*)	0.533 mg.	0.711 mg.	0.889 mg.	1.111 mg.
Food (*c*) plus water (*b*) or (*c*)	0.613 mg.	0.818 mg.	1.023 mg.	1.278 mg.
Food (*d*) plus water (*b*) or (*c*)	0.745 mg.	1.000 mg.	1.250 mg.	1.560 mg.
Food (*a*) plus water (*d*)	0.587 mg.	0.781 mg.	0.975 mg.	1.221 mg.
Food (*b*) plus water (*d*)	0.613 mg.	0.816 mg.	1.019 mg.	1.276 mg.
Food (*c*) plus water (*d*)	0.693 mg.	0.923 mg.	1.153 mg.	1.443 mg.
Food (*d*) plus water (*d*)	0.825 mg.	1.105 mg.	1.380 mg.	1.725 mg.

TABLE 4.—*Fluorine content of foods as reported in the literature* *

Food	Fluorine, Parts per Million	Food	Fluorine, Parts per Million
Fluorine Reported in Food as Consumed			
Milk	0.07-0.22	Pork chop	1.00
Egg white	0.00-0.60	Frankfurters	1.70
Egg yolk	0.40-2.00	Round steak	1.30
Butter	1.50	Oysters	1.50
Cheese	1.60	Herring (smoked)	3.50
Beef	<0.20	Canned shrimp	4.40
Liver	1.50-1.60	Canned sardines	7.30-12.50
Veal	0.90	Canned salmon	5.60- 9.00
Mutton	<0.20	Fresh fish	1.60- 7.00
Chicken	1.40	Canned mackerel	26.89†
Pork	<0.20		
Fluorine Reported in Dry Substance of Food			
Rice	<1.00	Honey	1.00
Corn	<1.00	Cocoa	0.50-2.00
Corn (canned)	<0.20	Milk chocolate	0.50-2.00
Oats	1.30	Chocolate (plain)	0.50
Crushed oats	<0.20	Tea (various brands)	30.00-60.00
Dried beans	0.20	Cabbage	0.31- 0.50
Whole buckwheat	1.70	Lettuce	0.60- 0.80
Wheat bran	<1.00	Spinach	1.00
Whole wheat flour	1.30	Tomatoes	0.60-0.90
Biscuit flour	0.00	Turnips	<0.30
Flour	1.10-1.20	Carrots	<0.20
White bread	1.00	Potato (white)	<0.20
Ginger biscuits	2.00	Potato (sweet)	<0.20
Rye bread	5.30	Apples	0.80
Gelatin	0.00	Pineapple (canned)	0.00
Dextrose	0.50	Orange	0.22

* For the source of these analytic data see McClure.[18]
† Lee and Nielson.[21]

Lawrenz and Mitchell (*18*) found that about 20 percent more of water-borne fluorine was assimilated than of fluorine ingested in food. This impairment of assimulation of fluorine due to the presence of food in the alimentary tract was observed when rigid control was exercised over the animals' habits of eating and drinking. This observed difference in assimilation of fluorine would need to be taken into consideration if similar conditions surrounded ingestion of fluorine by human subjects, as in case an allotment of fluorine in the diet was ingested entirely in the food or the fluorine-containing water was ingested on an empty stomach. Results obtained by me (*15*) relative to water-borne vs. food-borne fluorine, when no control was exercised over eating and drinking habits, gave no indications of a difference in total assimilation of fluorine in young rats. In this test the presence of food in the alimentary tract during any drinking period obviously obscured the effect of water-borne fluorine per se. These results also would be applicable only if the drinking habits of the rat and of man were similar. Considering the uncertainties surrounding individual drinking habits, a difference in the hygienic effect of fluorine ingested in food as compared with fluorine ingested in drinking water has not been applied to the figures given in table 3. In regard to the effects of fluoride in food alone the following facts may be noted: (*a*) Fluorine in water and fluorine in food both have inhibitory effects on experimental caries in rats (*19*); (*b*) endemic dental fluorosis (mottled enamel) appears to result from food-borne and water-borne fluorine (*20*); (*c*) Lee and Nielson found that fluorine contained in fish was assimilated by young rats (*21*), and (*d*) fluorine in tea is assimilated and produces characteristic effects experimentally (*22*).

The estimates of fluorine intake shown in table 3 may be compared with actual fluorine content of an average diet consumed by a man, as determined by Machle, Scott and Largent (*23*). The actual fluorine intake based on a 9 month study was approximately 0.45 mg. daily. The drinking water contained no fluorine, all the dietary fluorine being ingested in food and beverages. It was noted that almost twice as much fluorine was contributed by fluids (tea, coffee, milk, beverages and beer) as was present in the solid food. As would be expected, the consumption of iced tea during the summer seemed to account for a notable elevation in intake of fluorine.

In table 5 are summary estimates of fluorine intake such as appear in table 3. The quantity of fluorine ingested by children from drinking water containing 1 part per million of fluorine according to these estimates will equal approxi-

TABLE 5.—*Summary of estimated daily intake of fluorine from food and from drinking water (drinking water containing 1 part per million of fluorine and dry substance of food containing 0.1 to 1 part per million of fluorine)*

Age, Years	Body * Weight, Kg.	Daily Fluorine Intake			
		From Drinking Water, Mg.	From Food, Mg.	Total Daily from Food and Drinking Water, Mg.	Total Daily, Mg./Kg. of Body Weight
1 to 3	8 to 16	0.390-0.560	0.027-0.265	0.417-0.825	0.026-0.103
4 to 6	13 to 24	0.520-0.745	0.036-0.360	0.556-1.105	0.023-0.085
7 to 9	16 to 35	0.650-0.930	0.045-0.450	0.695-1.380	0.020-0.086
10 to 12	25 to 54	0.810-1.165	0.056-0.560	0.866-1.725	0.016-0.069

* Figures for weight for ages 1 to 6 years were taken from tables arranged by Woodbury (Woodbury, R. M.: Statures and Weights of Children Under Six Years of Age, Publication 87, U. S. Department of Labor, Children's Bureau, 1921); figures for 6 to 12 years were taken from the Baldwin-Wood weight-height-age tables for boys and girls of school age, published by the American Child Health Association.

mately 0.5 to 1 mg. of fluorine daily. This quantity of water-borne fluorine plus fluorine contained in foods may bring the total amount of fluorine contained in the average daily diet up to 1 to 1.50 mg. The ratio of these approximate levels of intake to body weight would probably rarely exceed 0.1 mg. per kilogram of body weight (table 5). As a general rule this average would equal about 0.05 mg. daily per kilogram of weight for children of these age groups.

SUMMARY

An estimated quantity of water-borne fluorine equal to approximately 0.5 to 1 mg. of fluorine daily present in the average diet from the first to the eighth year of life appears to be instrumental in reducing dental caries to a great degree. There has been no evidence presented heretofore to compare with this mass reduction of caries accompanying a modification of a dietary factor.

While conclusions may be modified by further experimental and clinical studies, at this time the evidence does not indicate that a health hazard surrounds this small quantity of additional fluorine ingested during the comparatively few years of formative tooth life. The importance of the preventive effects of fluorine on dental caries suggests that serious thought be given to the use of this optimum quantity of supplemental fluorine in children's diets for the partial control of dental caries.

In certain communities the drinking water may contain fluorine naturally in a quantity equal to 1 part of fluorine per million. In these places a supplement of dietary fluorine obviously would be unnecessary and undesirable. There is under consideration also the advisability of a direct fluorination of drinking water (*24*) so as to provide an optimum quantity of fluorine for improved dental health. Under this condition, likewise, the previous recommendation for additional fluorine in the diet would not apply. Therefore, only in communities in which the water supplies either are fluorine free or contain suboptimal amounts of fluorine need serious consideration be given to the possible use of a supplement of fluorine in children's diets. Calculations of the quantity of supplemental fluorine required must take into account fluorine present naturally in the local water supply.

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FLUORINE IN FOODS (Survey of Recent Data—1949)

By F. J. McClure

The analytical data relating to fluorine in foods have accumulated during the past decade to an extent that makes it desirable to assemble the data for purposes of comparison, as well as for an evaluation of the amount of fluorine ingested in the average human diet. With few exceptions, the more recent fluorine data are comparable as regards the analytical procedure since the Willard and Winter technique (*1*), or a slight modification (*2, 3, 4, 5*), has become the most generally utilized flourine method. While most analysts recognize the desirability of improvements in the fluorine determination (in view of an expected error of at least 10 percent, particularly in the analysis of organic materials), it has been some time since any radical changes have been made in the Willard and Winter procedure. Generally this fluorine method now calls for ashing the sample in the presence of an alkaline fixative at a low temperature, isolation of the fluorine by steam distillation using perchloric acid, and estimation of the fluorine in the distillate by microtitration with thorium nitrate or by comparative colorimetry.

Published results for fluorine in foods from various sources are compiled in table 1. In table 2, the data concern the relation of the fluorine content of soil and water to the fluorine present in plant produce. Similarly, data concerning the effect of fluorine ingestion on fluorine in animal produce (meat, eggs, and milk) appear in table 1, sections a, d, e. In several of the publications cited, information is lacking as regards the "dry" or "fresh" condition of the material analyzed, and wherever it seemed desirable to supply such information the judgment of the author was based on the analytical figure. In general, results for meats, fish, eggs, milk, and wine are based on the materials as consumed. Other materials are reported on a fresh or a dry-weight basis, or both.

TABLE 1.—*Fluorine in animal tissues, meats, fish, hen's egg, cow's milk, citrus fruits, noncitrus fruits, tea, cereals and cereal products, vegetables and tubers, miscellaneous substances and wine*

a. ANIMAL TISSUES

	Fluorine ppm
Cow's liver, dry wt. (*6*):	
No F added to ration	5. 50, 5. 80, 5. 20
F added to grain ration:	
.022 percent	7. 80, 8. 50, 5. 30
.044 percent	7. 30, 8. 30, 6. 20
.088 percent	8. 50, 7. 70
Chicken liver, fresh wt. (*7*):	
NaF injected:	
63 mg	0. 7015
45 mg	1. 209
90 mg	1. 291
45 mg	trace
30 mg	1. 010
30 mg	1. 131
Liver, fresh wt. (*8*)	1. 43
Do	1. 59
Do	1. 52
Guinea pig liver, fresh wt. (*12*)	0. 40
Calf liver, fresh wt. (*12*)	0. 19
Beef liver (*10*)	0. 99
Cow's kidney, dry wt. (*6*):	
No F added to ration	6. 9, 8. 9, 10. 1
F added to grain ration:	
.022 percent	31. 8, 25. 7, 25. 5
.044 percent	34. 7, 33. 5, 38. 2
.088 percent	43. 0, 43. 7
Guinea pig kidney, fresh wt. (*12*)	0. 06
Cow's pancreas, dry wt. (*6*):	
No F added to ration	6. 9, 10. 3, 8. 5
F added to grain ration:	
.022 percent	7. 0, 8. 2, 9. 5
.044 percent	8. 3, 9. 0, 9. 2
.088 percent	9. 1, 10. 6
Cow's heart muscle, dry wt. (*6*):	
No F added to ration	2. 3, 2. 7, 2. 7
NaF added to grain ration:	
.022 percent	4. 8, 3. 7, 8. 8
.044 percent	7. 5, 6. 4, 9. 7
.088 percent	9. 4, 8. 7
Guinea pig heart, fresh wt. (*12*)	0.24

b. MEATS

	Fluorine ppm
Chicken (*10*)	1. 40
Poultry, canned boned chicken (*11*)	0. 63
Beef (*26*)	2. 00
Round steak (*10*)	1. 28
Beef (*13*)	<0. 20
Beef, fresh wt. (*11*)	0. 29
Pork (*13*)	<0. 20
Pork, fresh wt. (*11*)	0. 34
Pork chops (*10*)	0. 98
Pork shoulder (*10*)	1. 20
Salt pork (*13*)	1. 1
Salt pork (*10*)	3. 33
Frankfurters (*10*)	1. 67
Lamb (*10*)	1. 20
Veal (*10*)	0. 90
Mutton (*13*)	<0. 20

c. FISH

	Fluorine ppm
Fish, fillets (*11*)	1. 49
Fish (*10*)	1. 63
Mackerel:	
boned (*13*)	<0. 2
with bones (*13*)	3. 9
fresh (*14*)	26. 89
dried (*14*)	84. 47
canned (*11*)	12. 10
Salmon:	
canned (*13*)	4. 5
red, canned (*15*)	8. 5
pink, canned (*15*)	9. 0
fresh (*14*)	5. 77
dried (*14*)	19. 34
canned (*11*)	4. 16
Sardines:	
canned (*13*)	7. 3
Sardines—Continued	
canned (*15*)	12. 5
in olive oil (*11*)	16. 10
Shrimp:	
canned (*15*)	4. 4
edible portion (*11*)	0. 93
Codfish:	
fresh (*15*)	7. 0
salted (*15*)	5. 0
Oysters:	
fresh (*15*)	0. 65
unspecified (*10*)	1. 58
unspecified (*15*)	1. 5
Crab meat, canned (*15*)	2. 00
Herring, smoked (*15*)	3. 50
Tuna fish flakes, canned (*11*)	0. 10

NOTE.—Italic numbers in parentheses are references. See pages 1073–1074.

The results for animal tissues appear in table 1–a. The effect of sodium fluoride ingestion on fluorine in kidney tissue is notable. To produce this result, however, there was a pronounced induced fluorine toxicosis in the animal. The major portion of fluorine retained in the animal body is deposited in skeletal tissue. As shown in table 1-b, the fluorine in normal edible cuts of meat is of the order of 0.2–0.3 p.p.m. or less fluorine.

Seafoods (table 1–c) are particularly interesting because they generally contain more fluorine than any other food, except tea, which obviously is not in a class with seafoods as an edible substance. Sea water may contain upwards of 1.2–1.4 p.p.m. fluorine (*9*) and is the source of fluorine in seafood. The amount of bone remaining, particularly in canned fish, no doubt determines to a major extent the quantity of fluorine contained in the product.

The amount of fluorine in the hen's egg (table 1–d) is approximately 0.2–0.4 p.p.m. The fluorine results recorded in the table were obtained during an experiment (*16*) in which rock phosphate was a source of fluorine in the hen's ration for a period of 28 months. There was a definite increase of fluorine in the egg yolk, the fluorine being present almost exclusively in the acetone-insoluble portion of the fat-like substance of the egg yolk.

The fluorine content of cow's milk has never proved to be affected by fluoride in the cow's ration or drinking water (table 1–e). Normally cow's milk contains 0.10–0.20 p.p.m. fluorine.

Fluorine in tea has been studied extensively (table 1–f). The data agree that tea is an unusual plant substance in its fluorine content. It has been reported that 75 percent or more of the fluorine in tea is extracted by boiling water (*18, 21*). It has been estimated also (*18*) that about 0.1 mg. of fluorine may be present in the hot water extract from one tea ball (2.5 gm. of tea contains 62.8 p.p.m. fluorine in the dry tea).

The data for fluorine in citrus fruit (table 1–g), with three exceptions, were obtained by Hamersma (*20*). These results, based on the fresh or edible material, indicate the presence of about 0.10 p.p.m. or less fluorine.

A number of noncitrus fruits (table 1–h) are also reported by Hamersma (*20*), the origin of most of his material being the Union of South Africa. In the United States, fluorine in apples has attracted much interest because of a presumed health hazard arising from a fluoride spray remaining on the apples (*24*).

The common cereals and cereal products have been analyzed extensively for fluorine (table 1–i) as would be expected for such important articles of the diet. The fluorine figures are quite variable, but for corn and wheat particularly and their edible produce, the values generally are extremely low, i.e., of the order of 0.10 or 0.20 p.p.m. or less fluorine in the fresh material.

The seemingly high fluorine results for several vegetables and tubers (table 1–j) may be questioned in some instances, because of the possibility of soil contamination. The majority of results on the fresh weight basis, however, are in fairly good agreement, i.e., 0.10 to 0.30 p.p.m. is about the average amount to expect.

A variety of results on a number of miscellaneous substances are shown in table 1–k. The majority of these materials, however, do not constitute a very important part of the average diet.

Fluorine in wine (table 1–l) has been studied recently by von Fellenberg (*12*), whose laboratory is in Switzerland, and by de Almedia (*35*). The results reported by de Almedia appear to be unusually high. Sodium fluoride formerly had some use in cleaning wine tanks and casks, but there is no hazard from this type of fluoride usage at this time. Although consumption of wine, particularly among Europeans and South Americans, may be unusually high, there is at present no knowledge that wine causes an unusual dietary fluorine intake.

EFFECT OF SOIL AND WATER FLUORINE ON PLANT FLUORIDE

The data in table 2 answer the question of the relation of fluorine in plants to the fluorine in the water and soil in which the plants grow.

With few exceptions, and these seem to apply mostly to roots and tubers, fluorine in plant produce is not readily affected by fluorine in the soil and local water. According to Bartholomew (*33*), nutrient solutions containing fluorine up to 10 p.p.m. may cause large increases of fluorine in cowpea roots, but the tops are increased in fluorine only when the quantity of fluorine in the roots is very large. Fluorine in wheat changed little when calcium fluoride was added to soil plots (*19*). In studies of control vs. fluoride plots (*19*), fluorine in beets and yams increased notably when an excess of sodium fluoride was applied to the soil. Fertilization of soil with phosphates and slags containing fluorine may increase fluorine in drainage waters (*29*), but plant fluoride was not increased (*29, 30*). As much as 2,300 p.p.m. fluorine was added to the soil in one experiment (*30*), whereas an average figure for fluorine in surface soils is about 292 p.p.m. (*28*). Results of analyses of grains and forage crops from fluoride areas frequently show unusually high fluorine concentrations, but this may be caused by contamination with soil dust. The evidence regarding soil fluoride and its effect on fluorine in plants shows generally a negative effect.

Although all evidence points to the contrary, the fluorine in local water sup-

TABLE 1.—*Fluorine in animal tissues, meats, fish, hen's egg, cow's milk, citrus fruits, noncitrus fruits, tea, cereals and cereal products, vegetables and tubers, miscellaneous substances and wine*—Continued

d. HEN'S EGG

(All analyses based on fresh weight)

	Fluorine ppm
Eggs (*10*):	
Whole	1.18
White	1.48
Yolk	0.59
Eggs (7):	
Hen No. 1 [1]	0.360, 0.368, 0.288, 0.165
Hen No. 2 [1]	0.294, 0.463, 0.441, 0.206
Egg (*8*):	
White	0.47, 0.32, 0.14, 0.00
Yolk	1.20, 0.42
Eggs, fresh (*12*)	0.15, 0.21, 0.22, 0.22, 0.42, 0.13, 0.20
Eggs, 1 dozen mixed (*11*)	0.12

	Fluorine ppm
No. F added to ration (*16*)):	
White	0.20, 0.30
Yolk	0.90, 1.20
F in ration (*16*):	
.035 percent:	
White	0.20
Yolk	1.80
.070 percent:	
White	0.20
Yolk	3.30
.105 percent:	
White	0.30
Yolk	3.00

[1] During 4- to 8-week period hens received intermittent intravenous injections of 30–90 mg. NaF.

e. COW'S MILK

No unusual fluoride in cow's ration or drinking water

	Fluorine ppm
Whole milk (*17*)	0.07, 0.09
Do	0.10, 0.15
Do	0.15, 0.17
Do	0.07, 0.22
Fresh milk (*10*)	0.38
Fresh milk, 1 qt. mixed (*11*)	0.09
Fresh milk (*12*)	0.55

	Fluorine ppm
Commercial milk (*18*):	
Washington, D. C	0.22, 0.22, 0.26
Washington, D. C	0.14, 0.16, 0.13
Urbana, Ill	0.30, 0.13, 0.11, 0.10, 0.10, 0.10

Fluoride above normal in cow's ration or drinking water

	Fluorine ppm
F in drinking water:	
8 ppm (*18*)	0.26, 0.39, 0.19, 0.17, 0.23, 0.26, 0.18, 0.40, 0.49, 0.28, 0.29, 0.40
0.2 to 500 ppm (*19*)	0.20, 0.30, 0.50, 0.30
0.2 to 495 ppm (*19*)	0.00, 0.30, 0.40, 0.40, 0.40, 0.30, 0.40, 0.30
1.4 ppm (*12*)	0.97, 0.72, 0.91

	Fluorine ppm
F added to grain ration (*17*):	
.022 percent	0.15–0.20
.044 percent	0.11–0.15
.068 percent	0.14–0.26

f. TEA

	Fluorine ppm
Tea (*21*):	
Imported Indian	38.1
Imported Ceylon	8.7, 9.5
Indian Ceylon blends	28.5
Clipper	13.1
Anhwei	91.8, 54.3, 122.6
Amoy	52.7
Ting-ku	178.8
"Doubly scented"	91.7
1st grade Hunan	60.3
Yunnan	49.7
Jessamine	83.5
Hangchow, best grade	37.5
Hangchow, second grade	93.5
Cheap mixed	398.8
Tea (*15*):	
English breakfast	66.0
Gun powder	67.0
Oolong	41.0
Tea (*22*):	
White tea	6.80
Song, chian	9.89
Asiang-pain	43.20
Red tea	67.07
Lung-ching	70.70
Szechuan	85.63

	Fluorine ppm
Tea (*20*):	
Pu-er	91.25
Kocha	7.8
Makha	3.2
Hankow	4.1
Toko	9.2
Ajax	11.9
Maza wattee	29.4
Rajah	33.1
Lyons	18.4
Five roses	25.6
Gifto	18.4
Liptons	18.3
Indona	19.3
Fargo	18.4
Tea, average of ten samples (*11*)	97.0
Tea, infusion—15 gm. of tea were treated with 1,000 cc. boiling water, steeped 10 minutes and strained (*11*)	1.19
Liptons yellow label (*18*)	53.5
Orange Peko (*18*)	62.8
Tea, infusion—0.122 mg. of fluorine was extracted from one tea ball of 2.55 gm. tea (*18*)	

Table 1.—*Fluorine in animal tissues, meats, fish, hen's egg, cow's milk, citrus fruits, noncitrus fruits, tea, cereals and cereal products, vegetables and tubers, miscellaneous substances and wine*—Continued

g. CITRUS FRUITS

	Fluorine ppm
Grapefruit:	0.36
edible portion (*10*)	0.36
fresh (*20*)	0.12
Lemon, fresh (*12*)	.028, .051, .174
Orange, edible portion (*10*)	0.34
Oranges (*20*):	
fruit, fresh	0.17–0.07
Brazil fruit, fresh	0.15
Brazil, peel, fresh	0.16
California, peel, fresh	0.18
Jamaica, peel, fresh	0.11–0.14

Oranges—Continued	Fluorine ppm
Palestine, fruit, fresh	0.04–0.15
Palestine, peel, fresh	0.11–0.15
Spanish, peel, fresh	0.07–0.13
Navel:	
Spain, fresh	0.15
S. Australia, fruit, fresh	0.06
S. Australia, peel, fresh	0.11
Pomelo (*20*):	
fruit, fresh	0.10–0.16
Florida, fresh	0.04–0.25
Florida, peel, fresh	0.13–0.25

h. NONCITRUS FRUITS

	Fluorine ppm Fresh wt	Fluorine ppm Dry wt
Apples (*10*)	----------	0.42
Apples (*12*)	0.052	0.21
Apples (*20*)	0.22	-------
Apples (*12*)	0.035	0.13
Apples (*8*)	0.92, 1.10, 1.15	-------
Apples (*8*)	1.32, 1.30	-------
Apples, fresh only (*19*)	0.34, 0.77, 0.83, 0.87	-------
Apricots (*20*)	0.22	-------
Apricots (*12*)	0.02	0.08
Apricots (*12*)	0.06	0.24
Banana (*20*)	0.23	-------
Banana (*10*)	----------	0.65
Cherry (*20*)	0.25	-------
Cherries, black (*12*)	0.18	0.61
Currants (*20*)	0.12	0.69
Fig (*20*)	0.21	-------
Grapes (*20*)	0.16	-------
Grape juice (*12*)	0.003	-------

	Fluorine ppm Fresh wt	Fluorine ppm Dry wt
Grape seed (*12*)	0.105	-------
Gooseberries (*20*)	0.52	-------
Gooseberries (*12*)	0.11	0.72
Guava (*20*)	0.34	-------
Mango (*20*)	0.18	-------
Pawpaw (*20*)	0.15	-------
Pear (*20*)	0.19	-------
Prickly pear (*20*)	0.26	-------
Pears (*10*)	----------	0.70
Peach (*20*)	0.21	-------
Plum (*20*)	0.21	-------
Plum (*12*)	0.22	0.10
Pineapple (*20*)	0.14	-------
Pineapple tinned (*26*)	0.00	-------
Pomegranate (*20*)	0.20	-------
Quince (*12*)	0.06	0.37
Quince (*20*)	0.20	-------
Sweet melon (*20*)	0.20	-------
Strawberry (*20*)	0.18	-------
Watermelon (*20*)	0.11	-------

i. CEREALS AND CEREAL PRODUCTS

	Fluorine ppm Fresh wt	Fluorine ppm Dry wt
Corn:		
unspecified (*22*)	0.62	0.70
unspecified (*25*)	----------	1.0–2.0
canned (*13*)	<.20	----------
canned (*10*)	----------	.42
unspecified (*22*)	----------	5.00
yellow (*13*)	<.10	----------
germ (*25*)	----------	8.0–11.0
germ (*13*)	----------	15.0
meal, as purchased (*11*)	.22	----------
meal (*25*)	----------	2.00
flakes (*10*)	----------	1.33
Ralston (*10*)	----------	.58
Wheat:		
whole (*22*)	----------	.53
unspecified (*21*)	----------	1.70
unspecified (*13*)	<.10	----------
unspecified (*27*)	.7	.8
bran (*27*)	.29	.33
bran (*13*)	<.2	----------
germ A, commercial (*13*)	1.7	----------
germ B, commercial (*13*)	4.0	----------
germ, pure (*13*)	<.1	----------
germ, pure (*27*)	.88	1.00
Cream of Wheat (*10*)	----------	.55
Flour:		
wheat, white (*22*)	.35	----------
self-rising (*11*)	.45	----------
whole wheat (*25*)	----------	1.32
white (*27*)	.27	.31
biscuit (*26*)	----------	.0
baking (*27*)	.31	.35
Bread:		
white (*11*)	----------	.54
white (*26*)	----------	1.00
white (*10*)	----------	.82
Biscuits (*26*)	----------	0.0–1.0
Do.	----------	2.0–2.0

	Fluorine ppm Fresh wt	Fluorine ppm Dry wt
Ginger biscuits (*26*)	----------	2.0–2.0–2.0
Rice:		
unspecified (*27*)	.67	.76
whole (*13*)	<.10	----------
unspecified (*21*)	----------	.70
middle (*22*)	.19	----------
unspecified (*32*)	----------	.80
Soy beans (*21*)	----------	4.00
Soy beans (*11*)	1.33	----------
Soy flour (*11*):		
low fat	----------	.52
with fat	----------	1.45
Buckwheat:		
unspecified (*34*)	----------	2.00
whole (*21*)	----------	1.70
bran (*21*)	----------	1.60
Millet (*21*)	----------	.20
Millet (*12*)	.60	.91
Oats:		
unspecified (*34*)	----------	3.0
crushed (*13*)	<.20	----------
Mother's (*10*)	----------	.92
fresh (*27*)	.26	.29
Rye:		
from Norway (*18*)	----------	.34, .04, 1.30
unspecified (*12*)	.61	.69
Blackeyed peas (*11*)	.23	----------
Chick peas (*11*)	.14	----------
Cottonseed:		
meal (*13*)	12.0	----------
meal (*25*)	20.0–31.0	----------
hulls (*25*)	12.0–14.0	----------
Spaghetti (*10*):		
canned	----------	1.15
dry	----------	.80
Macaroni, dry (*10*)	----------	.82

plies has been suggested frequently as influencing fluorine in plant life. Machle, Scott, and Treon (*10*) found no correlation between fluorine in certain food plants and fluorine in the local water supplies of Arizona. Wheat produced in Deaf Smith County, Tex., a fluoride water area, did not show an unusual fluorine content (*11*). Hamersma (*20*) has presented results regarding the effects of water containing 4.0–12.7 p.p.m. fluorine on vegetables produced in small private gardens. A number of his results are unusually high for fresh materials and a residue of fluoride on the plant materials is suggested by his notation that the garden was watered by hose. As in the case of soil fluoride, there is no indication that fluorine in the local water supplies affects food fluoride. Sources of fluoride-bearing potable waters are, with few exceptions, deep wells, and these waters are not used for irrigation purposes. It is not to be expected, therefore, that normal use of water in fluoride water areas would add fluoride to the local plant produce.

Fluorine may be increased in foods cooked in fluoride waters, as the following results presented by Smith, Smith, and Vavich (*19*) indicate:

Food	Cooked in distilled water pmm	Cooked in water containing— 5 ppm fluorine pmm	Cooked in water containing— 24 ppm fluorine pmm
Pinto beans	2.0	----------	37.1
Beets	0	1.0	----------
Potatoes	.5	----------	9.7
Cabbage	0	3.6	----------
Carrots	2.3	3.2	----------
Cauliflower	0	4.2	----------
Oatmeal	.9	----------	22.8
Spinach	2.0	4.0	----------
Italian squash	.2	3.8	----------
Brussel sprouts	.2	2.9	----------

ASSIMILATION OF NATURAL FLUORINE

The body's assimilation of "natural" fluorine in foods has been subjected to limited investigation. In addition to being largely extracted from a 2-percent infusion of tea leaves, the fluorine in tea, according to Reid (*21*), is capable of producing the characteristic striations in the incisor teeth of rats. Fluorine from various sources was administered at levels of 9–12 p.p.m. to rats by Lawrenz and Mitchell (*31*) who found that green-tea fluoride was only about 5 percent less well assimilated than was sodium fluoride or calcium fluoride. An average of 31.7 percent of the fluorine in green tea was retained as compared with 33.7 percent retention from sodium fluoride.

Results on the rats' metabolism of the fluorine contained in canned fish are reported as follows by Lee and Nilson (*14*):

Source of fluorine in rat's diet	F in diet ppm	Total F ingestion mg.	Percent stored
Salmon (fresh)	5.77	13.87	19.75
Salmon (dried)	19.34	12.10	20.25
Mackerel (fresh)	26.89	50.75	21.47
Mackerel (dried)	84.47	49.20	24.24

This percentage of fish fluoride body storage by the rat is somewhat low when compared with the usual retention of inorganic fluorides by the growing rat (*31, 32*), but the quantities ingested in this study (*14*) are also relatively high.

In general it appears that natural fluorine in fish, tea, and other foods is largely available for assimilation. This conclusion is indicated also indirectly by urinary excretion data mentioned later on in this discussion.

DISCUSSION

In a previous article (*36*) the total foodborne fluorine in the diets, exclusive of drinking water, of children 1–12 years old was estimated to be 0.25 mg.–0.55 mg. daily. Uncertainties surrounding food-fluorine analytical data at that time (1943) suggested that estimates of dietary intake should be based on 0.1, 0.2, 0.5, and 1.0 p.p.m. fluorine in the dry weight of average foods. The analytical fluorine data accumulated since then do not substantially change these quantities—0.25–0.55 mg. fluorine daily in food—although the lower values, 0.25–0.30 mg. fluorine in the daily food, exclusive of drinking water, are probably more representative and in accord with the analytical data for fluorine in foods. Other fluorine analyses of entire diets exclusive of drinking water have indicated 0.25–0.32 mg. fluorine in the average daily food alone (*37*). Another study indicates 0.45 mg. fluorine in the average diet (*38*) where the drinking water is practically fluoride free.

It may be pointed out that 0.25–0.30 mg. of dietary fluorine may be applicable to average daily diets throughout the United States and perhaps other parts of the world. Thus Machle et al. (*10*) and McClure and Kinser (*39*), studying the urinary excretion of fluorine, found the urinary fluorine analysis to be a valuable criteria of the daily water-borne fluorine intake. In widely scattered areas in the United States, where the drinking water contained only traces of fluorine, the urinary fluorine may be attributed largely to food-ingested fluorine and is quite uniform, i.e., 0.2–0.3 p.p.m. (*39*). This observation was regarded as indicative of a uniform content of fluorine in average daily diets, regardless of the locality. Similar urinary fluorine data were obtained recently with respect to Oslo, Norway, where the drinking water contains about 0.1 p.p.m. fluorine (*18*).

Disregarding certain extreme industrial exposures, it is generally true that drinking waters containing upwards of 1.00 or more p.p.m. fluorine are the source of the major quantities of dietary fluorine. As regards water containing 1.00 p.p.m. fluorine, it is estimated that 1.0–1.5 mg. fluorine (based on an estimated 1,000–1,500 cc. water consumed daily) are ingested daily by an average adult via drinking water and water added to cooked foods (*36*). In the case of children 1–12 years old, drinking water containing 1.00 p.p.m. fluorine will contribute an estimated 0.4–1.1 mg. fluorine daily above the fluorine in food (*36*). This added quantity of fluorine ingested during the crown calcification period of tooth life—through ages 8 to 10, or through ages 12 to 16 if the third molar teeth are to be considered—is the estimated amount of water-borne fluorine now associated with the partial alleviation of dental caries (*40*). The advantages to dental health surrounding the use of drinking water containing 1.00 p.p.m. fluorine has justified investigation of the dental health value of a direct addition of sodium fluoride to community water supplies (*41*) and has suggested also the direct addition of a fluoride supplement to children's diets during formative tooth life (*36*).

The importance of fluorine in preventive dentistry has been widely discussed and thus far remains irrefutable (*40*). Many problems, however, have yet to be resolved regarding the most efficacious utilization of fluorides in dental caries prevention. Where fluorides are not present in drinking water and cannot be provided via a community water supply, serious consideration seemingly may be given to the advantages of a direct dietary fluoride supplement. For purposes of dental health it appears that during formative tooth life the average child's diet should contain an additional dental optimum supplement of a fluoride equal to about 1.00 mg. of fluorine daily. The accumulated data on the fluorine content of foods indicate that the average

TABLE 1.—*Fluorine in animal tissues, meats, fish, hen's egg, cow's milk, citrus fruits, noncitrus fruits, tea, cereals and cereal products, vegetables and tubers, miscellaneous substances and wine*—Continued

j. VEGETABLES AND TUBERS

	Fluorine ppm *Fresh wt*	*Fluorine ppm* *Dry wt*
Aniseed (*20*)	0.4	--------
Amaranth (*21*):		
red	--------	8.5
green	--------	6.9
Asparagus, canned (*10*)	--------	0.48
Beans:		
string (*10*)	--------	0.64
string, canned (*10*)	--------	0.67
string (*11*)	0.13	--------
string, edible pods (*23*)	--------	3.2, 4.8
green (*12*)	0.15	1.01
light green (*12*)	0.11	0.73
lima, dry (*10*)	--------	4.51
lima, seeds (*23*)	--------	2.2
dry (*22*)	--------	1.04
dried (*13*)	--------	<0.20
navy, dry (*10*)	--------	1.70
Beets:		
unspecified (*20*)	0.2	--------
fresh (*10*)	--------	0.60
root (*21*)	--------	2.8
leaves, dry (*21*)	--------	3.80
tops (*23*)	--------	3.4
leaves (*12*)	0.38	3.45
roots (*23*)	--------	4.3
roots, sugar beet (*23*)	--------	3.3
string (*12*)	0.32	6.09
Cauliflower:		
fresh (*10*)	--------	0.45
flower (*12*)	0.12	0.86
leaves (*12*)	0.08	0.83
unspecified (*20*)	1.0	--------
Cabbage:		
large (*22*)	--------	9.34
foreign (*22*)	--------	15.38
fresh (*10*)	--------	0.70
unspecified (*22*)	0.13	--------
edible head (*23*)	--------	3.4
without leaves (*12*)	0.8	9.5
edible part (*12*)	0.15	1.5
unspecified (*20*)	0.3	--------
loose leaf (*12*)	0.38	1.31
loose leaf, stalk (*12*)	0.12	0.86
Carrots:		
unspecified (*20*)	0.4	--------
fresh (*10*)	--------	1.30
root (*23*)	--------	8.4
unspecified (*13*)	<0.20	--------
unspecified (*21*)	--------	6.92
Celery:		
unspecified (*22*)	--------	1.47
unspecified (*11*)	0.14	--------
powder (*11*)	9.10	--------
edible stalks (*23*)	--------	8.5
unspecified (*12*)	0.70	5.70
Cress (*12*)	0.24	4.38
Cucumber (*20*)	0.20	--------
Colza shoot, red (*22*)	--------	3.88
Colza (*22*)	--------	2.15
Eggplant (*20*)	0.4	--------
Endive (*20*)	0.2	--------
Garlic:		
green (*22*)	--------	17.72
unspecified (*22*)	--------	7.17
unspecified (*20*)	0.3	--------
Ginger plant (*22*)	--------	2.36
Kale (*11*)	0.16	--------
Kale (*20*)	3.0	--------
Leeks (*22*)	--------	3.90
Leeks (*20*)	0.1	--------
Lettuce:		
loose, head (*23*)	--------	11.3
cabbage (*12*)	0.30	4.45
prickly (*12*)	--------	5.18
fresh (*10*)	--------	0.42
Marjoram (*12*)	1.92	8.68
Mustard:		
greens (*11*)	0.15	--------
leaves, salted, dried (*21*)	--------	3.0–4.8
Onions:		
green (*22*)	--------	10.11
unspecified (*20*)	0.60	--------
unspecified (*12*)	0.24	2.40
Parsley:		
tops (*23*)	--------	11.3
unspecified (*20*)	0.8	--------
unspecified (*12*)	1.04	8.73
Parsnip, roots (*23*)	--------	5.5
Peas:		
unspecified (*20*)	0.6	--------
green (*21*)	--------	6.69
fresh (*10*)	--------	0.60
Potatoes:		
white (*10*)	--------	0.96
unspecified (*13*)	0.20	--------
whole (*17*)	6.4	22.0
peelings (*12*)	0.07	0.35
Irish, tuber (*23*)	--------	1.4
white, unpeeled (*22*)	0.16	--------
unspecified (*21*)	--------	0.73
unspecified (*26*)	--------	1.0
I, from Norway (*18*)	--------	0.9
II, from Norway (*18*)	--------	0.3
sweet, unpeeled (*11*)	0.13	--------
sweet (*13*)	<0.20	--------
sweet (*10*)	--------	1.08
Pumpkin (*20*)	0.10	--------
Radish (*20*)	0.8	--------
Rhubarb (*20*)	0.4	--------
Rutabaga:		
tops (*23*)	--------	7.0
roots (*23*)	--------	2.9
Spinach:		
fresh (*10*)	--------	1.11
unspecified (*20*)	1.8	--------
unspecified (*22*)	--------	7.97
unspecified (*11*)	0.21	--------
unspecified (*11*)	0.36	--------
unspecified (*23*)	--------	28.3
winter (*12*)	0.44	3.80
Squash, fresh (*10*)	--------	0.63
Shepherd's purse (*4*)	--------	2.26
Summer savory (*12*)	2.67	12.10
Tomatoes:		
unspecified (*12*)	0.24	2.40
fresh (*10*)	--------	0.53
fruit (*23*)	--------	0.0
Turnips:		
greens (*22*)	0.10	--------
tops (*23*)	--------	1.7
roots (*23*)	--------	2.6
unspecified (*13*)	<0.20	--------
unspecified (*20*)	0.30	--------
fresh (*10*)	--------	0.56
Watercress (*20*)	1.0	--------

Table 1.—*Fluorine in animal tissues, meats, fish, hen's egg, cow's milk, citrus fruits, noncitrus fruits, tea, cereals and cereal products, vegetables and tubers, miscellaneous substances and wine*—Continued

k. MISCELLANEOUS SUBSTANCES

	Fluorine ppm	
	Fresh wt	*Dry wt*
Peanuts:		
unspecified (*10*)	....	1.36
unspecified (*15*)	<0.20	....
tops (*25*)	....	1.7
kernel (*25*)	....	1.5
Almonds (*12*)	0.90	0.90
Hazelnut (*12*)	0.30	0.30
Chestnut (*12*)	....	1.45
shell (*12*)	....	0.24
Coconut, fresh (*11*)	0.00	....
Cocoa (*26*)	0.5, 0.50, 2.0	....
as purchased (*11*)	0.92	....
Plain chocolate (*26*)	0.50	....
Milk chocolate (*26*)	0.5, 1.0, 2.0	....
Molasses (*11*)	0.00	....
Sugar (*10*)	0.32	....

	Fluorine ppm	
	Fresh wt	*Dry wt*
Honey (*26*)	1.00	....
Gelatin (*26*)	0.00	....
Glucose (*26*)	0.50	....
Malt (*26*)	1.0, 1.5	....
Powdered ginger (*26*)	1.00	....
Baking powder (*13*):		
A	220.0	....
B	19.0	....
C	<0.1	....
Coffee (*21*):		
Mocha, Arabian	1.6	....
Del Monte, Brazilian	0.7	....
E. B. C., Brazilian	0.2	....
best raw Java	1.1	....
Butter (*10*)	1.50	....
Cheese (*10*)	1.62	....
Pork and beans, canned (*10*)	1.40	....

l. WINE

	Fluorine ppm
Chinese, Shao-shing (*21*):	
best grade	0.07
second grade	0.05
Chinese Lao, pai, chiu (*21*)	0.09
Port (*21*)	0.24
Beer (*11*)	0.20
Neuenberger (*12*)	0, 0, 0, 0, 0, 0.06, 0.08, 0.10, 0.10, 0.17, 0.18, 0.20, 0.24, 0.26, 0.34, 4.68, 6.34.
Walliser (*12*)	0, .03, .04, .07, 0.11, 0.12, 0.20, 0.21, 0.23, 0.23, 0.25, 0.25, 0.41, 0.47, 0.54, 0.54.

	Fluorine ppm
Franzosischer (*12*):	
Weisswein, Cote d'or	0.22
Rotwein, Burgunder	0.31
Rotwein, Cote du Rhone	0.21
Do	0.07
Do	0.10
Italienischer, Rotwein (*12*)	0.21
Spanisher, Weisswein, Xers (*12*)	0.21
Algerischer, Weisswein (*12*)	0.26
Wine (*35*):	
variously dated	3.8, 3.3, 2.4
1938–1941	4.1, 2.1, 3.3, 2.7, (slight trace) 4.1, 3.3.
white Sta. Barbara, 1942	3.3
red. Sta. Barbara, 1942	5.0

child's diet does not provide a dental optimum quantity of fluorine.

SUMMARY

A survey of recent analytical data for fluorine in foods has been compiled. The majority of foods found in the average diet contain from 0.2–0.3 p.p.m. or less fluorine in the food as consumed. Tea and seafoods are notable exceptions, the former containing upwards of 75 to 100 p.p.m. fluorine in the dry tea, whereas seafoods may contain 5–15 p.p.m. fluorine. Cow's milk contains about 0.1–0.2 p.p.m. fluorine. Fluoride added to the cow's ration or dinking water has no influence on the milk-fluoride. Fluorine in soil and water has little or no influence on the fluorine content of edible plant produce. Although the data are limited, it appears that natural food-borne fluorine is largely available for body assimilation.

Exclusive of drinking water, the average diet appears to provide 0.2–0.3 mg. of fluorine daily. However, it has been observed that an additional intake of fluorine during formative tooth life, via drinking water containing 1.00 p.p.m. or slightly more fluorine, is a distinct dental health advantage.

It is justifiable, therefore, to consider the possibility of a direct dietary fluoride supplement where the drinking water does not provide a dental optimum quantity of fluorine.

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TABLE 2.—*Fluoride content of plants grown in soil or water containing added fluoride (all figures are p.p.m. of fluorine)*

Reference 19 (dry weight):	*No fluoride added to soil*	*Calcium fluoride added to soil*
Corn	trace	trace
Hogari	0.00	2.1
Soybeans	.83	1.2
Wheat	.69	1.0
Wheat, stalks and leaves	3.70	7.2
Alfalfa:		
1st cutting	7.0	15.0
2d cutting	4.5	10.8
3d cutting	5.0	---------
4th cutting	6.0	11.3

Reference 19 (fresh weight):	*No fluoride added to soil*	*Sodium fluoride added to soil*		
		800 ppm	*1600 ppm*	*3200 ppm*
Beets	1.7	6.5	------	17.7
Carrots	1.0	3.0	0.4	1.3
String beans	.0	------	.0	-------
Yams	.0	7.6	------	8.2
Tomatoes	.7	.5	2.3	1.2

Reference 29 (air dry basis):	*No fluoride added to soil*	*Phosphate fluoride fertilizer added to soil*		
Wheat grain	0.82	0.94	0.99	0.32
Do	.30	.48	.46	.26
Oat grain	1.25	1.75	.70	1.66
Alfalfa hay Mixed hay Clover and timothy hay Oat straw Wheat straw Cowpea hay	No consistent or greatly increased fluorine content.			

	Mineral fluorides added to soil
Reference 34:[1]	
Lawn grass	No appreciable effect on fluorine content.

Reference 21:[2]	*No fluoride added to soil*	
	Fresh wt	*Dry wt*
Wheat	0.20	----------
Rice	0.90	----------
Barley	0.02	----------
Soybeans	0.67	----------
Cowpeas	0.43	----------
Green beans	0.17	----------
Green peppers	0.14	----------
Mustard leaves	----------	26.55
Turnip leaves, salted	----------	4.04
Tea, fresh[3]	1.75–7.8	----------
Tea, roasted	15.0–71.0	----------

[1] Fluorine a normal constituent of vegetation in study area (western Pennsylvania).
[2] Study area "near fluorine area" in China.
[3] Picked in later summer instead of usual springtime, suggesting increase of fluorine with age of leaves.

TABLE 2.—*Fluoride content of plants grown in soil or water containing added fluoride (all figures are p.p.m. of fluorine)*—Continued

Reference 11 (dry weight):[4]	*No fluoride added to soil*
Wheat	0.76–1.15
Wheat flour	0.50

Reference 20 (fresh weight):	*Fluorine in water for plants*[5]
Cabbage	6.4
Salad	12.1, 28.3
Salad seed	21.3
Mealies	<1.0, 0.7, 0.1
Beetroot	6.4, 3.1
Beetroot leaves	9.1, 0.6
Carrots	9.1, 5.4
Carrot leaves	6.9, 56.6
Tomatoes	2.8, 6.4, 1.8
Leeks	54.7
Orange leaves	25.7
Radish	12.0
Radish leaves	132.0
Guavas	2.1
Beans	2.8
Pumpkin	3.4
Pumpkin peel	<0.1

	Fluorine (ppm) in local water supply			
Reference 10:[6]	*0.0–0.8*	*1.0, 2.0, 3.0*	*3.1–6.0*	*6.1–18.0*
Squash, white	0.42, .36	0.07	0.11, 0.26	0.36, 0.36
Squash, green	0.32	0.08	0.13, 0.24	--------
Tomatoes, green	--------	--------	--------	0.41
Tomatoes, red	0.62	--------	0.31, 0.29, 0.16	0.47
Onions, green	--------	--------	0.72	0.25
Onions, white	--------	0.52, 0.51	--------	0.12
Beets	--------	0.65	0.53	0.32, 0.73
Carrots	--------	0.63, 0.43	0.72, 1.34	0.74
Beans, string	0.16	0.30	0.43	--------
Lettuce	--------	0.27	--------	--------
Potatoes	--------	--------	--------	0.81
Cucumbers	--------	--------	0.15	--------
Okra	0.20	--------	0.42	--------
Chard	--------	--------	0.85	--------
Turnip tops	--------	--------	0.69	--------

	Fluorine in nutrient solutions	*Fluorine ppm*	
Cowpeas (Reference 33):	*ppm*	*In roots*	*In tops*
NaF in nutrient solution	0.25	13.7, 58.0	0.0
	0.50	8.0, 13.7	0.0
	1.00	39.1, 60.0	0.0
	3.00	237.5	0.0
	3.00	550.0	8.0
	10.00	826.0	26.2
	10.00	1,086.0	40.0
CaF_2 in nutrient solution	0.25–0.50	0.0	0.0
	7.72	84.3	0.0
	7.72	78.3	11.0
Na_2SiF_6 in nutrient solution	0.25–0.50	0.0, 13.7	0.0
	1.00	37.4, 42.7	0.0
	10.00	1,970.0	415.0
	10.00	1,116.0	475.0

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[4] Wheat produced in Deaf Smith County, Tex.

[5] Water for plants contained 4.0–12.7 ppm fluorine. Study area in South Africa.

[6] Results are presumably on fresh-weight basis.

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THE USE OF FLUORIDES IN THE PRACTICE OF DENTAL MEDICINE

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In order for the dental profession to appreciate and practice "dental medicine" it is essential that each practitioner understand and employ some type of preventive dentistry. The restoration of dental tissues destroyed by disease and the arrest of progress of pathologic conditions known to exist cannot in the *fullest sense* be considered preventive dentistry. At the present time, the profession is offered a number of methods and techniques which give reasonable promise of preventing, at least in part, the inception of one of its major diseases, dental caries. It is the purpose of this paper to review briefly and evluate some of the scientific evidence suggesting the use of fluoride compounds for the control of dental caries.

The evidence on hand at the present time suggests at least three practical methods whereby fluorides may be used to prevent, in part, those processes associated with the development of carious lesions of the teeth. These methods are as follows: first, the fluoridation of fluoride-deficient public water supplies, increasing their fluoride content to an optimum level (1-1.5 p.p.m. F.); second, topical application of fluoride solutions to the erupted teeth; third, the addition of fluoride supplements to the daily diet. The successful application of these control methods demands a full understanding of the following principles: first, the most practical use of fluorides for the control of dental caries demands a complete change in the general philosophy concerning the treatment procedures consistent with furnishing a group population a good and safe water supply; second, the use of fluorides for the control of dental caries necessitates a change from the usual policy of treating an existing disease condition in an individual patient to employing prophylactic procedures on a group basis.

FLUORIDATION OF PUBLIC WATER SUPPLIES

In many of the earlier studies on the etiology of mottled enamel (dental fluorosis) it was suggested that this pathologic condition affected the disease dental caries (*1*, *2*). It was not until 1938, however, that a specific effect of fluoride-bearing waters on dental caries was demonstrated. At that time Dean (*3*) reported a lower prevalence rate for dental caries in areas where dental fluorosis was known to exist and where the public water supplies were known to contain excessive amounts of fluorides. The significance of the various levels of fluorides in water supplies as related to dental fluorosis had previously been demonstrated (*4*) and it was known that certain levels of fluoride could be tolerated without producing serious macroscopic evidence of dental fluorosis. With these established facts as a background, together with laboratory evidence suggesting that fluorine changed the caries process in experimental animals, a series of epidemiological studies were undertaken. The purpose of these studies was to establish whether or not the use of fluoride waters was beneficial to the teeth with respect to caries and whether or not such an effect would be discernible when the water-fluoride concentrations were within permissible limits (1–1.5 p.p.m. F.).

It is not considered essential to this paper to review in detail the results of these different studies. A complete review was recently made by Dean (*5*) to which the reader may refer for specific details. A summary of the results of these studies is shown in table I.

As illustrated in table I, there is a definite correlation between the fluoride content of domestic water supplies and the prevalence of dental caries among the users of these waters. All children included for these studies had used the

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Table I.—*Summary of dental caries findings in 7,257 selected white school children, aged 12 to 14 years, in 21 cities of 4 states in relation to the fluoride (F.) content of the public water supply*

CITY AND STATE	NO. CHILDREN EXAMINED	CLINICAL					WATER			
		% OF CHILDREN EXAMINED* WITH LACTOBACILLI IN SALIVA IN AMOUNTS SPECIFIED NO. PER C.C.		PER CENT OF CHILDREN EXAMINED CARIES FREE	NO. PERMANENT TEETH, PER CHILD WITH DENTAL CARIES EXPERIENCE (D.M.F.)		FLUORIDE (F.) CONCENTRATION OF PUBLIC WATER SUPPLY IN P.P.M.		TOTAL HARDNESS IN P.P.M.	SOURCE
		NEG. AND LESS THAN 100	30,000 AND OVER							
Galesburg, Ill.	273			27.8	2.36		>1.4	1.9	247	Deep wells
Colorado Springs, Colo.	404			28.5	2.46	2.44		2.6	27	Surface (Pikes Peak)
Elmhurst, Ill.	170	35.7	24.7	25.3	2.52			1.8	323	Deep wells
Maywood, Ill.	171	39.6	20.9	29.8	2.58			1.2	75	Deep wells
Aurora, Ill.	633	38.2	25.6	23.5	2.81	2.94		1.2	329	Deep wells
East Moline, Ill.	152			20.4	3.03		1.0-1.4	1.2	276	Deep wells
Joliet, Ill.	447	35.6	26.6	18.3	3.23			1.3	349	Deep wells
Kewanee, Ill.	123			17.9	3.43			0.9	445	Deep wells
Pueblo, Colo.	614			10.6	4.12	4.10	0.5-0.9	0.6	302	Arkansas River
Elgin, Ill.	403	21.2	33.2	11.4	4.44			0.5	103	Deep wells
Marion, Ohio	263			5.7	5.56			0.4	209	Deep wells
Lima, Ohio	454			2.2	6.52			0.3	223	Surface, impounded
Evanston, Ill.	256	15.4	41.8	3.9	6.73			0.0	131	Lake Michigan
Middleton, Ohio	370			1.9	7.03			0.2	329	Deep and shallow wells
Quincy, Ill.	330			2.4	7.06					
Oak Park, Ill.	329	18.2	43.8	4.3	7.22	7.40	<0.5	0.1	88	Mississippi River
Zanesville, Ohio	459			2.6	7.33			0.0	132	Lake Michigan
Portsmouth, Ohio	469			1.3	7.72			0.2	291	Deep wells
Waukegan, Ill.	423	14.9	54.6	3.1	8.10			0.1	80	Ohio River
Elkhart, Ind.	278			1.4	8.23			0.0	134	Lake Michigan
Michigan City, Ind.	236			0.0	10.37			0.1	220	Deep wells
								0.1	141	Lake Michigan

From Dean, H. T., Jay, P., Arnold, F. A., Jr., and Elvove, E.: Pub. Health Rep. 56: 761-792, 1941; Dean, H. T., Arnold, F. A., Jr., and Elvove, E.: Pub. Health Rep. 57: 1115-1179, 1942.

*Estimate made only on children of 8 suburban Chicago communities.

respective water supplies continuously, from birth, 30 days in any calendar year being excepted. It should also be noted that an optical percentage decrease in caries rates was observed in areas where the fluoride content was in the range of 1 to 1.5 p.p.m. of fluorine. Little, if any, increased benefit to the permanent teeth is demonstrated by the use of waters containing more than this amount. Other studies in the United States and many foreign countries have confirmed these observations (*6*). Thus, we can conclude that the use during the first 14 years of life of domestic water supplies naturally containing "permissible" amounts of fluorides (1–1.5 p.p.m. F.) will materially reduce the amount of dental caries experience of any group of individuals.

These results, together with considerable laboratory evidence (7), have led to the assumption that the disease dental caries can be partially controlled in group populations by the addition of optimum amounts of fluorides to public water supplies. On the basis of such an assumption, many communities in this country have proceeded to add fluoride compounds, generally sodium fluoride, to their public water supplies. Preliminary results in some of these areas indicate that such fluoridation of water supplies will produce results similar to those observed in communities using natural fluoride waters (*8, 9*). However, for a full evaluation of this method of caries control it is necessary to follow the results of this procedure over a period of years concomitant with the formation and subsequent exposure of teeth to the risk of dental disease.

In recognition of the demand for an economical control procedure and realizing the time required for complete evaluation, the Board of Directors of the American Water Works Association has adopted a general policy which *in brief* is as follows:

> In communities where there is strong public demand and the procedure has the full approval of the local medical and dental societies, local and state health authorities, and others responsible for communal health, the water departments may properly participate in a program of fluoridation of the public water supplies. The procedures are divided into two categories: (a) *experimental*, where the fluoride-dental caries hypothesis is being subjected to verification under acceptable scientific methodology; (b) *empirical*, where the community provides the cost of fluoridation for the purpose of giving anticipated protection to

the teeth of the oncoming generation of children at the earliest possible time while awaiting the outcome of controlled experiments. This policy may well serve as a guide to the dental profession when faced with the problem of advising communities in regard to fluoridation of their water supplies.

Topical Application of Fluorides

More or less as a sequel to the early studies demonstrating the effect of fluoride waters on dental caries came the results of numerous studies showing the effects of topically applied fluorides. Laboratory and animal experimentation suggested that the effect of fluoride-waters on dental caries could be due to an increased fluoride content of the enamel. It was observed that the fluoride content of powdered enamel could be increased by exposure to solutions of fluoride salts. Preliminary reports (*11*, *12*) on small groups of individuals indicated that topical fluoride applications produced beneficial effects. In 1943, Knutson and Armstrong (*13*) reported on an adequate number of experimental subjects and demonstrated a definite beneficial effect by topical fluoride applications. This study was continued for a period of 3 years (*14*) and the results obtained over this period are shown in table II.

The data demonstrated a very significant reduction in the incidence of dental caries in those teeth which had been treated with the fluoride solution. These results have been confirmed by other workers and a recommended technique was published in the *Journal of the American Dental Association* (*15*). A brief summary of the experimental results up to 1948 may be gained from a statement by Galagan and Knutson (*16*):

> . . . the effects of topically applied fluoride on dental caries experience indicate that under the conditions of the application technique employed: (1) A series of four topical applications of a 2 percent solution of sodium fluoride, preceded by a single dental cleansing, effects a 40 percent reduction in dental caries incidence. More than four application do not increase the caries-prophylactic effect. (2) The caries-inhibiting value of topically applied sodium fluoride is not appreciably decreased during a 3-year period following treatment. (3) The omission of dental cleansing prior to a series of applications reduces the effectiveness of topically applied sodium fluoride solution by approximately half. (4) Application of a saturated solution of lead fluoride (0.07 percent), using the same application technique as for solutions of sodium fluoride, is not associated with a significant reduction in the incidence of dental decay.

Table III.—*Summary of estimated daily intake of fluorine from food and from drinking water containing 1.00 p.p.m. of fluorine and dry substance of food containing 0.10 to 1.00 p.p.m. of fluorine*

AGE IN YEARS	BODY WEIGHT (KG.)*	DAILY FLUORINE INTAKE			
		FROM DRINKING WATER (MG.)	FROM FOOD (MG.)	TOTAL DAILY FROM FOOD AND DRINKING WATER (MG.)	TOTAL DAILY MG./KG. OF BODY WEIGHT
1 to 3	8 to 16	0.390-0.560	0.027-0.265	0.417-0.825	0.026-0.103
4 to 6	13 to 24	0.520-0.745	0.036-0.360	0.556-1.105	0.023-0.085
7 to 9	16 to 35	0.650-0.930	0.045-0.450	0.695-1.380	0.020-0.068
10 to 12	25 to 54	0.810-1.165	0.056-0.560	0.866-1.725	0.016-0.069

From McClure, F. J.: Am. J. Dis. Child. 66: 362, 1943.

*Weight figures for ages 1 to 6 years taken from tables arranged by Woodbury, R. M.: Children's Bureau, United States Department of Labor; figures for ages 6 to 12 years, taken from the Baldwin—Wood weight—height—age tables for boys and girls of school age, published by the American Child Health Association.

Dietary Supplements of Fluorides

On the basis of the present evidence that an increased fluoride consumption via water produces a resistance to caries, it can be assumed that the same effect will be brought about by the addition of fluoride salts to the daily diet. Scientific evidence of the effectiveness of any such procedure is lacking at the present time. McClure (*17*) compiled data with respect to the quantities of fluoride present in foods and drinking water and estimated the quantities of fluorine which would be ingested daily in drinking water containing optimum levels of fluorides (table III). In that report he suggested as a possible control measure direct dietary supplements of fluorine. This control method was later discussed by Arnold (*18*) and can be made a practical procedure in areas where fluoride waters are not available.

On the basis of present evidence it may be suggested that the dietary fluorides be increased by a daily supplement of approximately 0.5 mg fluorine during the first 2 or 3 years of life and that this supplement be increased to 1.0 mg. fluorine for the remainder of the formative period of the teeth (8–9 years of age excluding the third molars). At the present time, a limited study of such a procedure is being carried out at the National Institute of Dental Research but significant data will not be forthcoming for another 2 or 3 years. So far, on the few individuals whose erupted teeth were calcified under such a regimen of fluoride supplements, there is no evidence of fluorosis. This would be the first manifestation of any chronic toxicosis.

Table II.—*Percent less new caries experience in the fluoride-treated mouth quadrants (left) than in the untreated quadrants (right) of a selected group of Minnesota children*

YEAR	UPPER JAW	LOWER JAW	BOTH JAWS
PER CENT LESS NEWLY CARIOUS TEETH			
1942-1943	46.0	30.3	39.8
1942-1944	43.9	37.4	41.4
1942-1945	36.6	36.8	36.7
1943-1944	48.2	43.8	46.6
1944-1945	22.6	21.6	22.2
PER CENT LESS NEWLY CARIOUS SURFACES IN PREVIOUSLY CARIOUS TEETH			
1942-1943	14.0	11.1	12.4
1942-1944	21.7	24.6	23.1
1942-1945	28.2	19.3	23.9
1943-1944	18.6	34.4	25.2
1944-1945	36.8	28.3	33.1

From Knutson, J. W., and Armstrong, W. D.: Pub. Health Rep. 61: 1683, 1946.

Mechanism of Fluoride Effect

An adequate explanation for the action of fluorides in preventing dental caries has not been demonstrated. The preponderance of evidence comes from the epidemiological studies regarding the effects of fluoride water supplies. This evidence is clear-cut in regard to the beneficial effect of using natural fluoride waters from birth to at least 14 years of age, and Deatherage (*19*) and McKay (*20*), together with others, have shown that the beneficial effect of fluorides is still demonstrable in adult life. Weaver's interpretation of his findings (*21*) is not in full accord with these findings. Studies at Bauxite, Arkansas (*22*), and in Illinois (*19*) present evidence which suggests that the period of tooth formation is the most important period of fluoride exposure. For example, individuals who had used fluoride waters during the formative period of their teeth retained their "resistance" to caries even when exposed to a fluoride-free environment. However, there is some evidence (*19*) to suggest that the "resistance" is somewhat lowered when an individual moves out of the fluoride area.

The effects from the opposite changes in environment, e.g., moving from a fluoride-free environment to a fluoride environment, are not so clear-cut. Studies at Maywood, Illinois (*18*), on a limited number of individuals indicated that there was no benefit to be gained at least after the first four or five years of life. Klein (*23*) and Deatherage (*24*) presented evidence suggesting that there is a beneficial change in caries incidence when an individual moves into a fluoride area from a fluoride-free area. Russell (*25*) interpreted his results as showing that the ingestion of fluorides only during tooth formation and prior to eruption does not change the caries incidence of these teeth. He also presented evidence (*26*) of limited character suggesting that it is important to have a fluoride exposure for at least some period following eruption of the teeth in order to obtain the beneficial effects. The need for further research to answer these questions is quite evident.

It has often been assumed that the decreased prevalence of caries in fluoride areas was associated with an increased fluoride content of the tooth. Probably the results reported by Armstrong and Brekhus (*27*) regarding an increased fluoride content of enamel of sound teeth promoted this hypothesis. However, the results obtained more recently by McClure (*28*) suggest that this difference in fluoride content is not consistent for the individual teeth. He suggested that an optimum level of fluorine in dental tissues will be associated with caries resistance, but this fluoride level will be a characteristic of the entire dentition and not limited to individual sound teeth alone.

The manner of action of topically applied fluorides on dental caries is, at present, equally as difficult to explain as the effects of water-borne fluoride. From the standpoint of laboratory experiments one might assume that the fluoride ion is absorbed onto the tooth surface structure. When so incorporated into the surface structure, the resistance of the enamel to acid decalcification is increased. At the present chemical analyses have failed to demonstrate any increase in fluoride content of treated "whole" teeth. Electron diffraction studies of treated surfaces (*29*) have shown a deposit of CaF_2 salts on the surfaces. The permanency of such deposits is questionable at present. Further studies of this type may possibly give us some clue as to a surface effect of a permanent nature.

The results of studies where topically applied fluorides have been used are quite consistent in those cases in which the experimental techniques are comparable. In carrying out the technique as described by Knutson (*10*), about a 40 percent decrease in caries incidence has been obtained. Some reports have shown negative results or much less beneficial effects (*6, 30, 31*). It should be emphasized, however, that in these studies variations in solutions, age groupings, and method of application were not similar to the technique used in the successful experiments. Such conflicting results do suggest the need for further research in order to explain the action of the fluoride solutions.

One of the criticisms of the use of topical fluorides in the practice of dentistry has been to the effect that at present we cannot select the particular patient on whom to use the procedure. It is difficult to determine from published data whether or not the 40 percent reduction resulting from topical applications occurred in greater degree in only a small portion of the experimental subjects, having little effect on the majority of patients. Knutson (*32*) presented evidence, however, suggesting that the inhibition of carious lesions occurs in the large majority of the treated cases. Without doubt some patients who present themselves at a dental office will not experience further carious lesions over a reasonable future period. To hesitate, on such an excuse, about using a caries-preventive procedure which is as little involved as topical fluoride applications is not practicing *preventive dental medicine*.

As stated previously, the basis for the use of fluoride supplements to the diet is the epidemiological evidence regarding the beneficial effects associated with the use of fluoride water supplies. There is sufficient evidence on which to calculate a daily prophylactic dose (*17*). As pointed out by Arnold (*33*), the procedure was recommended many years before the hazards of fluorides were known. Today we have sufficient knowledge to conclude that such levels of fluorine in water as are necessary to prevent dental caries would not constitute a hazard from adverse physiological effects. Since the dosages used in dietary supplements are based on average water consumption, we can assume the same degree of safety. Whether or not the intermittent high dosages of fluorides will bring about similar results as have been observed when the same total amounts of this element are ingested over a period of hours is still to be demonstrated. One report on a fairly large group of individuals in which the results are admittedly inconclusive (*31*) suggested that fluoride "medication" may produce beneficial dental effects.

SUMMARY

Adequate presumptive evidence from epidemiological studies and laboratory experimentation suggests that dental caries can be partially controlled by fluoridation of domestic water supplies. It may be concluded in the light of present knowledge that such a procedure will not be associated with adverse physiological effects. The full evaluation of the benefits of employing this method of caries control must await the final results of experimental studies now in progress.

It has been adequately demonstrated on the basis of clinical trials that the topical application of fluorides to the teeth of children will materially reduce the incidence of caries. Variations in technique, types of solutions to be used, and individual susceptibility are some of the factors needing further study.

Fluoride medication in the form of supplements of this element to the daily diet are suggested for use in those cases in which fluoridation of domestic water supplies would be impractical. Such a procedure should be carried out during the formative period of the crowns of the teeth, i.e., birth to 8 or 9 years of age, third molars excluded.

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A SYSTEM FOR FLUORIDATING INDIVIDUAL WATER SUPPLIES

Franz J. Maier

Our aim should be to make the benefits of fluoridated water available to everyone in the United States. At the present time persons who live in communities served by a public water supply constitute about 60 percent of our total population (*1*). Consequently, about 60 million persons living in 20 million homes are being served by private, individual water sources—principally wells. It is likely that fluoridated water from a public supply never will be available to almost one-third of the people of this country. The proportion appears to be increasing slightly each year as more people move to suburban and rural areas not yet served by public water supplies.

Many methods have been suggested for providing fluorides for this group. At the present time the most widely used method is the topical application of fluorides. By this means a relatively strong fluoride solution is applied to children's teeth. This treatment must be done by a dentist or dental hygienist in the dentist's office or in clinics. The cost is considerably more than the fluoridation of water supplies and the results obtained are in the order of a 40 percent reduction in dental caries as compared to 60–65 percent for fluoridated water. As a public health measure it has not proved effective principally because of the difficulty in providing treatment on a widespread basis.

The use of bottled fluoridated water has been suggested. The cost of this is also much more than that of fluoridated water from a public supply and the distribution of bottled water in a rural area would be very complicated. In addition, the method of dispensing the bottled water in the home requires special equipment and does not guarantee that children will use this source consistently, particularly when other sources are available.

The use of pills containing fluoride has been tried in several areas, but the costs appear excessive as compared with fluoridating public water supplies. As a public health measure the procedure has not been effective principally because of the prolonged effort required on the part of parents to provide the pills invariably, constantly, and persistently. In addition, the hazards associated with a supply of toxic materials in the home cannot be ignored. There is often the inclination to increase the recommended dose slightly in order to insure the anticipated results. Other suggested methods, such as the distribution and use of fluoridated salt, baby foods, vitamin pills, and milk have similar drawbacks. All known experience and information indicate the method involving the least hazard with the greatest proved effectiveness is the fluoridation of the existing water supply.

From the standpoint of fluoridation an individual, home water supply, as compared to the fluoridation of a municipal supply, has these characteristics: the extremely small amounts of water pumped and consumed; the very wide variations in types of installations, some of which are of such primitive design that they probably never could be automatically fluoridated successfully; the operating personnel generally are not trained in water treatment technics; and the proper maintenance of the private water system is a chore which is sometimes successfully avoided.

To fluoridate a private water supply safely and effectively a device had to be developed which was capable of adding minute quantities of fluorides automatically to small water supplies. When such a device was found, then a system had to be evolved so that this device could be operated and maintained effectively and continuously and with no hazard to the consumer.

The criteria which were considered in such a program included: utmost safety from the point of view of avoiding overdosage; consistent fluoride levels within the error of 0.1mg/1; a device to prevent tampering of the feeding equipment; periodic surveillance to assure constant dosage and to provide maintenance; and replenishment of the fluoride solution.

A device which in part satisfied these criteria was developed and has been used during the past 2 years in four homes in a suburban area of Maryland. Each of these homes is served by individual wells, approximately 3 inches in diameter, delivering between 3 and 9 gallons of water per minute by means of jet

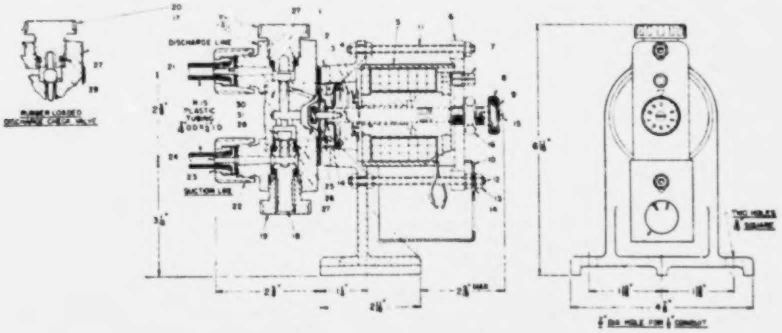

Figure 1.—*Solenoid-actuated diaphragm feeder.*

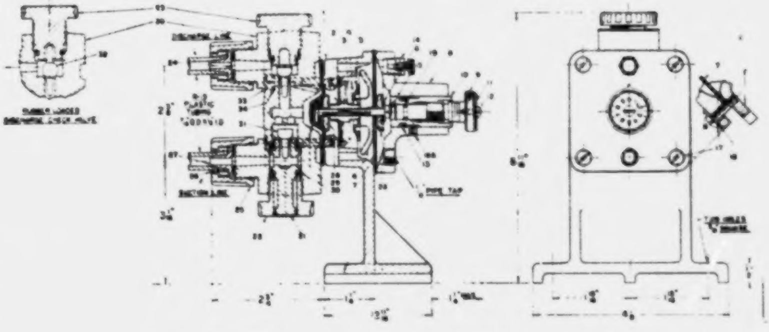

Figure 2.—*Water pressure-actuated diaphragm feeder.*

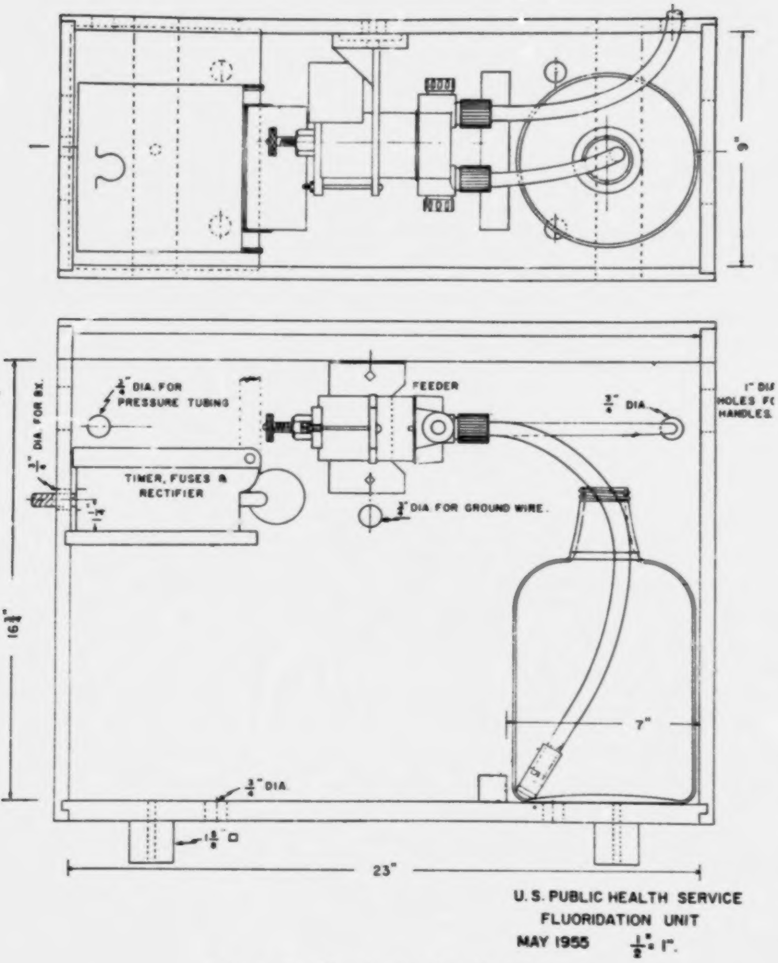

FIGURE 3.—*Housing for fluoride feeder.*

pumps. Each pump discharges to a hydropneumatic tank and thence through a water meter to the system. A vacuum relief valve is installed on a line between the tank and distribution system. Pressure switches automatically start and stop the well pumps (and/or feeders) at preset pressure levels.

The two types of feeders used are commercially available. They were selected primarily because they could be controlled at very low rates of discharge. Other types probably can be used just as successfully.

One type of feeder, figure 1, is actuated by a solenoid which is energized periodically through a rectifier by means of an electric timer. The timers used with this type of feeder operate at either four or 10 contacts per minute, although timers are available commercially for a variety of speeds.

The other type of feeder, figure 2, is hydraulically operated, the driving energy being derived from the water pressure in the distribution system. The feeding diaphragm of this feeder is actuated by another, larger diaphragm by means of the water pressure released by a solenoid-operated valve on the water line. This solenoid is energized by a timer running at either four or 10 contacts per minute. The spent water from the driving diaphragm is piped back to the suction side of the well pump.

Both types of feeders are housed in locked boxes which contain the timing and rectifying equipment and a glass container holding one gallon of fluoride solution (fig. 3). These feeding devices are designed for homes using electrically driven well pumps which discharge into a pressure tank. At present, they cannot be used in homes without electricity or without a pressure water system, such as those that have wells equipped with bucket and windlass, endless chains, pitcher- and other hand-operated pumps. However, other types of feeding devices probably can be developed to be used under some of these conditions.

Various fluoride compounds were used to make up solutions of different concentrations. Solutions of hydrofluosilicic acid, ammonium silicofluoride, sodium fluoride, and magnesium silicofluoride were used in strengths ranging from 0.5 to 2.6 percent as fluoride ion. It was found that "fortified" hydrofluosilicic acid (acid to which a small amount of HF is added by the manufacturer to prevent deposition of colloidal silica) (*2*) could be made up most easily, in that no solubility problems are encountered. Under some conditions this diluted acid is excessively corrosive and for that reason magnesium silicofluoride, which is soluble up to 65 percent, was used. It was also learned that the best strength is that which can be handled succesfully by the feeder in terms of the minimum practical rate of delivery. The stronger the solution, on the other hand, the longer the intervals between replenishments. In addition, the stronger solutions (greater than 1.0 percent) are bactericidal.

The units are installed near the well pump as shown in figure 4. The timers are wired into the pressure switches so the feeders operate only when the well pumps are running. The discharge from the feeders is injected into the distribution system ahead of the hydropneumatic tank in order to provide an opportunity for mixing and for minimizing variations of fluoride concentration due to pulsations in feeding.

During most of the period of testing the units were serviced each month, although from the point of view of replenishing solution once each three months would have been sufficient in most instances. During each visit the feeders are tested, fluoride solution used is measured and replenished, necessary repairs are made, water samples are taken for fluoride analysis, and water consumption is noted. Before installation of the equipment and occasionally thereafter samples of the untreated water were taken for fluoride analysis. The water consumption in the homes involved has varied between 151–580 gallons per day.

The feeders generally operated satisfactorily. Minor repairs had to be made occasionally involving fuse replacement, repair of leaks, or replacement of plastic parts, all of which affected the continuity of feeding. However, none of these factors affected the safety of the procedure from the point of view of overdosing. The delivery of fluoride solutions at the

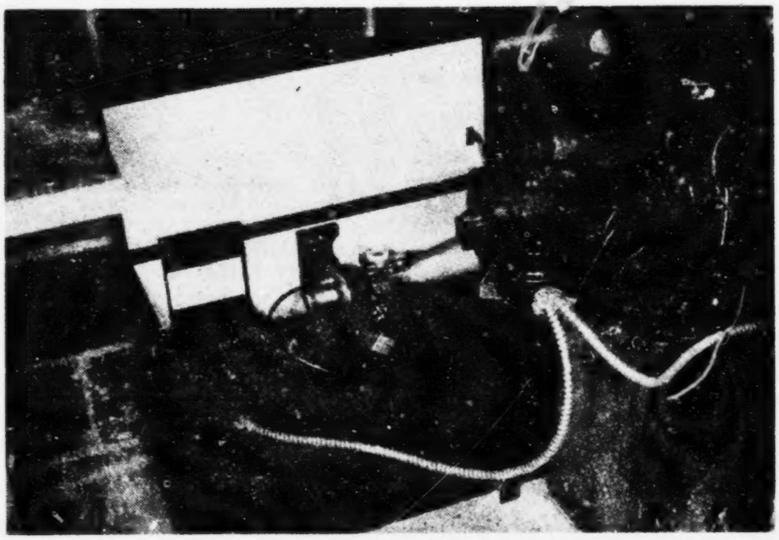

Figure 4.—*Fluoride-feeding device installed in basement.*

settings chosen is remarkably constant and precise. In most instances random samples of fluoridated water show a fluoride concentration well within 0.1 mg/1 fluoride as compared to the calculated fluoride content based on gallons pumped against milliliters of solution delivered during the same period. We did not attempt to operate the feeder at rates below 0.12 ml. per stroke because of the difficulty in adjusting the feeder at lower flows. At this setting, however, the accuracy was found to be within 0.1 mg/1. During one four-month period, for example, the analysis of four samples showed a maximum variation of only 0.02 mg/1 fluoride. This accuracy, of course, may change as the diaphragms become older, but at this point the feeders have not operated long enough to reveal a loss in accuracy.

There does not appear to be any difference in the ability to adjust or in the accuracy of the electrically-actuated feeders compared with those operated hydraulically. However, the hydraulic model seems to operate more smoothly, probably because the action of the solenoid is dampened by the fluid cushioning of the driving diaphragm. With the hydraulic model there is no possibility of the feeder operating if the pump switch is on but no water is being delivered.

Experience with this feeder design indicates that a satisfactory fluoride level can be achieved in home water systems if the installations are properly maintained and adjusted periodically. Despite the performance of any such devices, however, there must be available a service or system which will assure their continued satisfactory operation. To assure continuity of such service which should include the expert maintenance of the equipment, the preparation and replenishment of fluoride solutions, and a constant surveillance of the resulting fluoride concentration in the treated water, the householder should probably be relieved of all responsibility.

These objectives might be achieved by employing a service group organized to provide fluoridated water to owners of private water systems. The householder would purchase a service through which he could obtain at his existing faucets fluoridated drinking water maintained constantly at the optimum level. To do this a servicing organization similar to those now providing chlorinated water, softened water, or bottled water would supply and maintain the fluoridation equipment and be responsible for the entire installation.

These organizations would analyze the untreated water, provide, install, and maintain the feeding equipment, periodically inspect the installation, replenish the fluoride solution and check the dosage, and eventually remove the equipment when no longer needed.

In certain areas where conditions warrant the service might be provided by the local health department. In any case the quality of the service and the safety of the procedure should be the immediate concern of the health department.

A cost estimate for this service might be made as follows:

	Cost per Year
Amortization of equipment—30 years	$ 5.00
Interest	3.50
Repairs and installation	2.00
Fluoride solution	0.45
Labor (assuming 6,000 visits per year per man with each installation visited 6 times per year)	5.00
Laboratory services (equivalent of one sample per month)	0.35
Overhead	2.00
Contingencies, profit, etc.	18.00
Total	$36.30

Based on these assumptions it may be possible to provide this service at a charge of approximately $3 per month. The rate would vary greatly, however, depending on the number of customers on a route, how close together the customers were grouped, and the efficiency with which the units were serviced. At the end of the period when continued fluoridation of a particular supply would confer relatively fewer benefits (absence of children, advancing age of children, and so forth) the machine could be removed, rehabilitated, and used again in another home.

In addition, a service organization using the same equipment could contract to chlorinate these water supplies for consumers. The feeders described might be used for feeding chlorine and fluoride solutions simultaneously. By calculating the quantity of hypochlorite or other source of chlorine to be added to the gallon jug of fluoride solution the optimum quantity of fluoride ion and chlorine might be supplied.

Feeding devices of other designs and those for treating water only at a faucet can be developed for this purpose. Nevertheless, whatever form a feeder may take, a service organization to perform essentially the tasks outlined above is indispensable. The system suggested is practicable and with good management may be profitable to entrepreneurs experienced in service of this kind. In areas with no public water supplies it is believed that such a system can confer the maximum protection against tooth decay at the least cost.

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SODIUM FLUORIDE TABLETS FOR CHILDREN

By Francis A. Arnold, Jr., F. J. McClure, and Carl L. White

An accepted method of controlling tooth decay is by drinking water that contains, either naturally or by design, optimum quantities of fluoride (*1*). There are situations, however, in which some other way of adding fluoride to the diet would be more convenient, and from time to time various methods of doing this have been suggested (*2*). The one that has received the most serious consideration and study is the daily consumption of a tablet containing fluoride. A number of studies have been made that indicated that some degree of caries prevention resulted from the use of sodium fluoride or calcium fluoride tablets, but the evidence offered in these reports has been rather limited (*3–6*).

A report by Wrzodek (7), in Germany, presented more recent data on the cariostatic effects of sodium fluoride tablets. The results of this study, even by a preliminary evaluation, are quite extensive and provocative. Fluoride tablets were given to 13,585 school children for a period of 4 years. During this time they were observed and compared with a group of 4,975 children who did not take any tablets. Inhibition of caries intensity averaged approximately 20 percent for first-, second-, and third-grade children who had taken the tablets.

The fluoride-tablet regime followed in the study reported here differed in some important ways from those followed in previous studies. First, some of the children began to take fluoride soon after birth, and the majority of them began to use fluoride tablets before age 6. Second, these children were a rather select group, because their parents were physicians, dentists, or other professional employees of the Public Health Service who wanted their children to have the best dental care, and these children and parents were considered capable of following instructions consistently and exactly. With a few exceptions, all families co-operating in the study lived in the Washington, D.C., area when they started the program.

METHOD

Previous evaluation of drinking water consumed by children indicated that water containing about 1.0 p.p.m. fluoride would give the average child 0–3 years old approximately 0.4–0.6 mg. daily, in addition to the 0.2–0.3 mg. fluoride provided daily by the average diet (*8*). This was, in general, the basis on which the supplementary fluoride was added.

At the start of this study parents were given concentrated solutions of sodium fluoride and told to add a quantity of this solution equivalent to 1.00 mg. of fluoride to the child's total daily drinking water. After 2 years it was decided that it would be wiser and more effective to find some method of distributing fluoride that would reduce variation in individual measurement and also be more convenient. The solution was replaced by tablets containing 2.21 mg. of NaF (1.0 mg. of fluoride).

Because it was important to be sure that the total amount of fluoride each child took daily did not exceed a safe maximum, home water supplies whose fluoride contents were unknown were analyzed before additional fluoride was started. Whenever a family moved, which happened frequently, their new water supply was analyzed unless its fluoride content was known. These analyses were done in the laboratories of the National Institute of Dental Research, where the study was made.

A three-month supply of tablets was given to each family, and they were told to ask for more when the original supply was nearly gone. Instructions for the use of these tablets were:

Children 0 to 2 years old: Makeup all water added to any formula and used for drinking purposes, by dissolving 1 tablet in 1 quart of water.

Children 2 to 3 years old: Give 1 tablet every other day. This tablet may be taken directly with water, or dissolved in a glass of water, milk or fruit juice. To be certain the tablet is completely dissolved, place it in a glass with a tablespoon of water, crush and stir until it is completely dissolved. Milk, fruit juice or water may be added to make a full glass. Stir the liquid.

Children 3 to 10 years old: Give 1 tablet daily as directed above for 2–3-year-old children.

CAUTION: Do not increase these amounts. While sodium fluoride is not toxic at the levels prescribed, it is toxic in higher concentrations. These tablets should *not* be used where the local domestic water supply contains more than 0.50 p.p.m. of fluoride, or where the water is being directly fluoridated for control of dental caries. Keep tablets away from children.

Parents were asked to send their children's deciduous teeth to the Institute for fluoride analysis as soon as they were exfoliated. Several deciduous teeth from any one child had to be collected and pooled to obtain enough material for an analysis. Sufficient teeth were obtained from thirteen children. Mr. W. A. Lee, of the laboratory of biochemistry of the Institute, analyzed the teeth for fluoride and the results were plotted against the mean number of years they had been exposed to supplementary fluoride. The length of exposure is an estimate based on the interval between the date of the first issuance of the fluoride supplement and the mean of the receipt of each exfoliated tooth in the analytic pool.

Most of the dental examinations of children in the Washington area were made at the National Institute of Dental Research. The parents of children who lived in other areas were given definite instructions and a standard record form to be filled in by the examining dentist. Examination records were available for 141 of the 221 children who received tablets for one year or more. Twenty of these were under four or over fifteen years of age and were not included in the comparisons, so the data that follow were gathered from a statistical population of 121 children.

RESULTS

Nearly two-thirds of the children began to take fluoride tablets before their third birthdays and almost all before their sixth birthdays (table 1). The age and sex distribution of the children was nearly equal, and the group was not large; therefore subgroups of boys and girls were formed, and the data obtained for each individual in these groups were combined. There were 13 children in the 4 to 5 age group; 56 in the 6 to 9 age group; 31 in the 10 to 12 age group; and 21 in the 13 to 15 age group. The terms "def" (decayed, scheduled for extraction, filled) for deciduous teeth and "DMF" (decayed, missing, filled) for permanent teeth were used in recording the results of this study. Mean numbers of def and

Table 1.—*Distribution of children*

Beginning Age	No. Years of Ingestion of Fluoride Tablets													
	1	2	3	4	5	6	7	8	9	10	11	12	13	15
<1	4	3	5	3	6	9	7	3	2	4	..	1	1	..
1	2	5	3	3	2	3	5	1	..	2	2	1	..	..
2	..	2	1	4	1	3	2	..	..	..	2	..	..	1
3	..	2	4	2	1	..	3	3	..	2	2	..	..	..
4	..	1	1	1	1	2	3	..	..	..	1	..	..	..
5	1	2	2	..	.	1	3	3	..	..	1	..	..	..
6	1	..	..	1	1	..	..	..	..	..	..	..	..	..
7	..	..	..	..	..	..	1	..	..	..	..	..	..	..
8	..	..	..	..	..	..	1	..	..	..	..	..	..	..
9	..	..	..	..	..	..	..	..	..	..	..	..	..	..
10	..	..	..	..	..	..	..	..	..	..	..	..	..	..
11							1	..	..	..	..	..	..	..
	8	15	16	14	12	18	26	10	2	8	8	2	1	1

DMF teeth for these subgroups are shown in table 2 and are presented graphically in figures 1 and 2, with comparative data for children living in Grand Rapids, Michigan, before water fluoridation; children with 10 years' use of fluoridated water in Grand Rapids (*9*), Brantford, Ontario (*10*), and Newburgh, N.Y. (*11*); and children of Aurora, Ill. (*9*), with lifetime use of natural fluoride water.

Table 3 shows the results of fluoride analyses of whole deciduous teeth, compared with findings for similar teeth (supplied by Dr. Leonard Menczer, chief, Preschool Dental Health Program, Hartford, Conn.) from children using a nonfluoride water. A statistical test of the difference in the mean fluoride content in both methods of reporting the analyses of the teeth, with and without fluoride exposure, is significant at the 0.01 level.

Dental fluorosis was observed in only 4 of the 32 children examined at the institute. These cases were all classified as questionable except one, which was very mild. No fluorosis was reported in children examined by other dentists. While the examination form did not specifically request information about fluorosis, the examining dentists were aware of the exposure of the children's teeth to fluoride.

DISCUSSION

Evidence of the cariostatic effect of daily consumption of fluoride tablets in this study is necessarily presumptive, since the data cannot be compared directly with data obtained from a control group. However, it is obvious from table 2 that the cariostatic effect of the tablets on the children in this study is comparable to that experienced by children who have used community water containing approximately 1.0 p.p.m. of fluoride from birth. Other caries inhibitory or accelerative influences were not precluded in the design of the study.

TABLE 3.—*Fluoride contents of human deciduous teeth*

INGESTION OF TABLETS (YEARS)	ASH (PER CENT)	FLUORINE IN	
		Oven Dry (Per Cent)	Ash (Per Cent)
0*	74.95	0.0050	0.0066
0*	79.36	0.0044	0.0056
1.58	82.06	0.0054	0.0066
2.58	85.50	0.0061	0.0071
4.50	85.78	0.0049	0.0057
5.25	77.13	0.0037	0.0047
5.42	82.68	0.0101	0.0121
5.75	81.02	0.0124	0.0153
6.17	79.18	0.0082	0.0104
6.50	79.60	0.0177	0.0222
6.67	81.35	0.0066	0.0082
6.75	78.50	0.0191	0.0243
6.75	81.29	0.0158	0.0195
8.25	83.07	0.0095	0.0114
8.50	84.57	0.0065	0.0078

* Deciduous teeth from a non-fluoride area.

Some of the children were exposed to fluoridated water before their final dental examinations, and when this happened, use of the tablets was stopped. This fluoride-water exposure during the last few years of study (29 percent of the cases) could not have accounted for the results shown in table 2. Neither of the other studies shown in table 2 and figures 1 and 2 nor a recent study made in Maryland (*12*) report a decrease in caries of this magnitude after three to 5 years' use of fluoridated water.

The data as shown in table 3 suggest a trend toward increased amounts of fluoride in the whole tooth with length of time of consumption of fluoride tablets. The significant differences in the means of the fluoride content of teeth exposed to fluoride when compared with those not exposed to fluoride show the same characteristic which McClure and Likins (*13*) found for permanent teeth. Gdalia and Yardeni (*14*) reported an increased concentration of fluoride in sound deciduous teeth when increased amounts of fluoride were taken.

It seems highly probable from this study that fluoride taken daily in tablet form has a beneficial effect on the teeth, and such a program might be used in places where children do not have access to fluoridated drinking water. However, the fluoride content of the home water supply must be known. Experience gained during this study also suggests that a tablet regimen could be used effectively in controlled programs supervised by health authorities, such as school health programs. It is doubtful, however, whether any large-scale or community-wide program of caries control would be successful if tablets were used. Although the persons involved in this study were, on the whole, a highly educated group, only about half of them actually continued to give their children tablets for the necessary number of years.

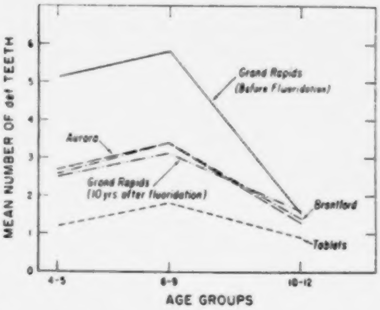

FIGURE 1.—*A comparison of the cariostatic effects of sodium fluoride tablets, fluoridated water, and non-fluoridated water on deciduous teeth.*

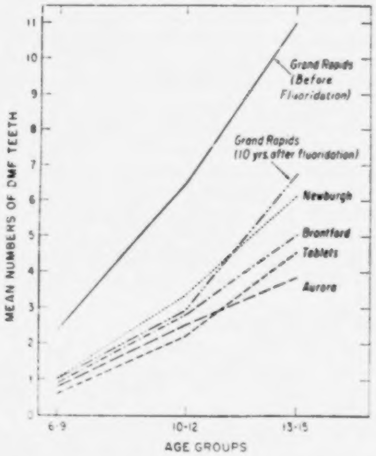

FIGURE 2.—*A comparison of the cariostatic effects of sodium fluoride tablets, fluoridated water, and non-fluoridated water on permanent teeth.*

TABLE 2.—*Mean number of DMF and def teeth of children who ingested NaF tablets and data reported from other studies, after 10 years of fluoridation and after use of a natural fluoride water**

AGE GROUPS	NAF TABLETS		AURORA, ILLINOIS*		GRAND RAPIDS, MICHIGAN†				BRANTFORD, ONTARIO†	
					Base Line		10 yrs. of F			
	def±SE	DMF±SE	def	DMF	def	DMF	def	DMF	def	DMF
4–5	1.2±0.6	...	2.7	...	5.2	...	2.5	...	2.6	...
6–9	1.8±0.4	0.6±0.2	3.4	0.8	5.8	2.3	3.1	1.0	3.4	0.9
10–12	0.9±0.3	2.2±0.5	1.3	2.5	1.5	6.5	1.6	2.9	1.4	2.8
13–15	...	4.5±0.9‡	...	3.8	...	11.0	...	6.7	...	5.0

* Aurora—a natural fluoride water.

† Grand Rapids and Brantford—fluoridated water.

‡ Five of this group were caries-free.

SUMMARY

The mean numbers of def and DMF teeth of 121 children who took a fluoride tablet containing 1.0 mg. fluoride as sodium fluoride every day for an average of two-thirds of their lives, were comparable to def and DMF data previously reported for children of similar ages who drank natural fluoride water or fluoridated water.

The regimen of daily fluoride tablets as followed in this study did not result in dental fluorosis of any cosmetic significance. The results correspond with what has been observed in the use of drinking waters containing 1 p.p.m. fluoride.

The mean fluoride content of deciduous teeth of children taking the fluoride tablets was significantly higher than the mean fluoride content of teeth of children in a nonfluoride area. It also appeared that the fluoride content of deciduous teeth increased slightly and proportionally with the length of time the sodium fluoride tablets were taken.

Although this study indicates that fluoride tablets seem to be an effective means of control, water fluoridation remains a more practical and dependable procedure for caries control in large population groups. (The reader's special attention is called to the report of the Council on Therapeutics, American Dental Association, J.A.D.A., *56*: 589–91, Apr. 1958.)

ACKNOWLEDGMENTS

Sharp & Dohme, Inc., supplied the sodium fluoride tablets used in this study.

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TOPICALLY APPLIED FLUORIDE AND DENTAL CARIES CONTROL

The oral surfaces of teeth acquire a resistance to dental caries if treated topically with a 1.0 or 2.0 percent solution of sodium fluoride. A series of four topical applications effects an approximate 40 percent reduction in the dental caries incidence. This caries inhibitory effect did not decrease appreciably during a 3-year period.

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THE EFFECT OF TOPICALLY APPLIED SODIUM FLUORIDE ON DENTAL CARIES EXPERIENCE

By JOHN W. KNUTSON and WALLACE D. ARMSTRONG

INTRODUCTION

The results of numerous investigations into the relationship between fluorine and dental disease led to the hypothesis that the incidence of dental caries could be reduced by topical applications of a fluoride solution to the teeth. Since there is an extensive literature on the subject of fluorine and dental caries, only the five major links in the chain of evidence which engendered and supported this hypothesis will be enumerated here: First, Armstrong and Brekhus (*1*) demonstrated that intact enamel of carious teeth contains less fluorine than enamel of noncarious teeth. Second, the epidemiological studies of Dean and his collaborators (*2, 3, 4, 5*) showed that the presence of approximately one part per million of fluoride in domestic water supplies is associated with a decreased prevalence of dental caries. Third, the results of animal experimentation demonstrated that the incidence of dental caries in the teeth of rats was reduced by the addition of extra fluorine to the diet (*6, 7, 8, 9*). Fourth, Volker, Hodge, Wilson, and Van Voorhis (*10*) discovered that fluorine is absorbed from solution by powdered enamel in accordance with the requirements of the Freundlich adsorption isotherm. Fifth, the fluorine content of the enamel of mature erupted rat molars is increased by giving drinking water containing 20 p.p.m. of fluoride and apparently the increase is brought about by direct adsorption (*11*).

Two preliminary reports made in 1942 presented the results of investigations into the effect of topical applications of fluoride to the teeth. Bibby (*12*) reported that during the year following the institution of treatment, 95 new carious surfaces occurred in the permanent teeth of the treated quadrants and 135 new carious surfaces occurred in the permanent teeth of the untreated mouth quadrants of 89 children aged 10 to 13 years. Treatment consisted of topical applications of a 0.1 percent solution of sodium fluoride to the teeth at intervals of 4 months. In reporting on a 3- to 3.5-month period of caries experience in the deciduous teeth of 27 treated children and 19 control children aged 4½ to 6 years, Cheyne (*13*) concluded that the treatment effected a 50-percent reduction in caries incidence and had a pronounced tendency to arrest lesions which were actively carious at the time of treatment. Treatment consisted of two complete applications of a 0.5 percent potassium fluoride solution to the teeth of the treated group. Recently Bibby (*14*) reported that the order of differences noted in the first preliminary report on his study group had decreased slightly 2 years after treatment was initiated. Although these pieces of direct evidence are not conclusive, they are in accord with the original hypothesis.

It is the purpose of this paper to present data on the incidence of dental caries during the year ended May 1943, in the permanent teeth of two groups of children. The first of these, a treated group consisting of 289 children, received topical applications of 2 percent sodium fluoride solution to the teeth in the upper left and lower left quadrants of the mouth. The second, a control group consisting of 326 children, did not receive the fluoride treatments. Briefly, the analysis of the data indicates:

1. In the treated group there were 39.8 percent less new carious teeth in the treated than in untreated teeth.
2. The difference between the caries incidence in the treated and untreated teeth was appreciably greater in the upper than in the lower teeth.
3. The number of new carious surfaces in teeth previously attacked by caries was not significantly different in the treated and untreated teeth.
4. The incidence of caries in the teeth of the right or untreated mouth quadrants of the treated group was not significantly different from that in the teeth of the right (or the left) quadrants of the control group.

MATERIAL AND METHODS

In March 1942, parental permission was obtained in three small urban centers in Minnesota to make topical application of fluoride to the teeth of 337 children aged 7 to 15 years. Of this group of children, 126 attended the public grade school in North Mankato, 114 attended either the public or parochial school in Arlington, and 97 attended junior high school in St. Louis Park.

Prior to the institution of treatment, each child in the treated group received a dental prophylaxis treatment consisting of scaling and polishing of the teeth and a detailed dental examination. The examinations were made with mouth mirror and explorer under artificial light and with compressed air available for use at the examiner's discretion. Only the teeth in the upper left and lower left quadrants of the mouth were treated. The treatment consisted of isolation of the teeth with cotton rolls, drying the teeth with compressed air, and wetting the crown surfaces of the teeth with 2 percent sodium fluoride solution. The applied solution was allowed to dry in air for approximately 3 minutes. After the cotton rolls had been removed the child was instructed to expectorate and he was then dismissed.

During an 8-week period in April and May 1942, the North Mankato and Arlington children in the treated group were given two treatments weekly to a maximum of 15 and a minimum of 8 treatments. The St. Louis Park children were given one treatment weekly to a maximum of 8 and a minimum of 7 treatments. The number of treatments given each child was determined largely on the basis of the maximum number of treatments that could be administered conveniently during the 8-week period remaining in the school year. This study was not designed to determine the minimum number of effective treatments but to assess the worth of relatively frequent exposures of the teeth to soluble fluorides administered over a relatively short period of time.

Children enrolled in the same schools as the treated group but not participating in the treatment program were utilized as a control group. The 392 children in this group were given dental prophylaxis treatment and dental examination similar to that given the children in the treated group.

Approximately 1 year after the fluoride treatments had been administered, the teeth of the children in both the treated and control groups were reexamined. The second examinations were made by the same person and with the same equipment as the first. Of the 337 children originally in the treated group, 289 were reexamined; of the 392 children originally in the control group, 326 were reexamined. Thus 14.2 percent of the treated group and 16.8 percent of the control group were not reexamined. Most of the children not available for the second examination had changed residence or were not attending school at the time the reexaminations were conducted. A few were absent from school.

TABLE 1.—*The incidence of caries in permanent teeth previously free of caries. A comparison of fluorine-treated and untreated quadrants in each jaw of 289 treated children*

	Upper jaw	Lower jaw
	Persons	*Persons*
Lower incidence in treated quadrant	63	48
Higher incidence in treated quadrant	26	30
Incidence equal (one or more) in treated and untreated quadrant	15	12
No new carious teeth in either treated or untreated quadrant	185	199
Total number of children	289	289

Only the data pertaining to the children who were given a second examination are presented here. The analysis is confined to the dental caries experience in the erupted permanent teeth present at the time of the first examinations. The term *new caries* is used in this presentation to designate teeth or tooth surfaces which were noncarious in April 1942 and became carious (decayed or filled) during the year ended May 1943. A total of 14 teeth, 6 from the left and 8 from the right mouth quadrants, were extracted from children in the treated group during the study year. Since the new caries experience in these teeth prior to extraction is not known, they are omitted from the tabulations. The age classification refers to the age at the time of the first examination.

FINDINGS

Results obtained from a comparison of the incidence of new carious teeth in the treated and untreated mouth quadrants of each child in the treated group are summarized in table 1. Because of the bilaterally equal occurrence of dental caries in the teeth of population groups, it is reasonable to expect that the number of instances in which individuals have more caries in the left than in the right quadrants of the jaw would be equal to the number of instances in which the opposite obtains. From an inspection of the data in table 1, it will be noted that for the upper quadrants 63 children had fewer new carious teeth in the treated than in the untreated quadrants, whereas the relationship was in the reverse order in only 26 children. For the lower jaw, 48 children had fewer and 30 children had more new carious teeth in the treated than untreated quadrant.

A complete test of the statistical significance of the difference between the observed distribution of caries experience by mouth quadrants as given in table 1 and a theoretical distribution based on bilaterally equal occurrence of dental caries is possible provided the detailed data on dental caries in the individual teeth of each quadrant are available. However, a conservative estimate can be obtained readily by limiting the comparison to the instances in which there were less new carious teeth in the treated than in the untreated quadrants and the instances in which the reverse result was observed. On the assumption that these two events should occur with equal frequency, the probability that the observed differences would occur by chance is 0.0001 for the upper quadrants and 0.0417 for the lower quadrants.

The number of permanent teeth without evidence of past or present caries at the time of the first examination in 1942, and the number of these that were attacked by caries during the year ended May 1943, are presented for the treated group in table 2. In addition, the number of carious tooth surfaces in newly attacked teeth and the number of new carious surfaces in previously attacked teeth are also given.

It will be noted from the totals for

TABLE 2.—*Treated group, dental caries experience during the year ended May 1943, for the permanent teeth in the treated and untreated quadrants of the jaws of 289 Minnesota children*

Age	Urban center	Number of children	Treated					Untreated					Treated					Untreated				
			Upper left					Upper right					Lower left					Lower right				
			P teeth[1]	New DF teeth[2]	DF surfaces in new DF teeth[3]	New DF surfaces in DF teeth[4]	Total new DF surfaces[5]	P teeth[1]	New DF teeth[2]	DF surfaces in new DF teeth[3]	New DF surfaces in DF teeth[4]	Total new DF surfaces[5]	P teeth[1]	New DF teeth[2]	DF surfaces in new DF teeth[3]	New DF surfaces in DF teeth[4]	Total new DF surfaces[5]	P teeth[1]	New DF teeth[2]	DF surfaces in new DF teeth[3]	New DF surfaces in DF teeth[4]	Total new DF surfaces[5]
7	North Mankato	10	15	0	0	0	0	16	0	0	0	0	21	1	1	3	4	21	2	2	2	4
8	North Mankato	18	30	4	4	1	5	35	5	5	1	6	37	1	1	2	3	40	2	3	0	3
9	North Mankato	26	73	4	5	3	8	80	9	9	3	12	84	2	3	5	8	83	4	4	3	7
10	North Mankato	22	52	4	4	4	8	62	10	11	6	17	80	1	1	5	6	77	4	6	4	10
10	Arlington	19	54	2	2	1	3	51	3	3	0	3	66	3	4	4	8	71	2	3	3	6
10	Total	41	106	6	6	5	11	113	13	14	6	20	146	4	5	9	14	148	6	9	7	16
11	North Mankato	21	75	3	3	3	6	77	4	4	3	7	89	3	4	1	5	94	4	5	8	13
11	Arlington	18	63	4	4	0	4	63	4	4	4	8	85	4	6	4	10	83	4	4	6	10
11	Total	39	138	7	7	3	10	140	8	8	7	15	174	7	10	5	15	177	8	9	14	23
12	North Mankato	9	41	1	1	0	1	41	2	2	2	4	46	0	0	0	0	48	1	1	1	2
12	Arlington	26	116	5	7	8	15	112	13	16	2	18	127	3	4	6	10	131	15	15	6	21
12	St. Louis Park	16	60	3	3	2	5	57	2	2	3	5	80	4	4	4	8	70	2	3	4	7
12	Total	51	217	9	11	10	21	210	17	20	7	27	253	7	8	10	18	249	18	19	11	30
13	Arlington	15	78	2	4	6	10	82	10	10	3	13	81	3	3	5	8	78	3	3	6	9
13	St. Louis Park	29	120	8	8	4	12	124	17	20	5	25	150	10	11	8	19	158	12	14	10	24
13	Total	44	198	10	12	10	22	206	27	30	8	38	231	13	14	13	27	236	15	17	16	33
14	Arlington	19	81	6	7	8	15	81	8	8	4	12	98	3	3	0	3	95	5	6	4	10
14	St. Louis Park	31	114	6	8	7	15	105	11	12	18	30	142	6	8	16	24	149	6	6	10	16
14	Total	50	195	12	15	15	30	186	19	20	22	42	240	9	11	16	27	244	11	12	14	26
15	Arlington	3	16	1	2	0	2	15	0	0	1	1	16	1	1	0	1	16	0	0	2	2
15	St. Louis Park	7	22	1	1	2	3	25	2	3	2	5	34	1	2	1	3	32	0	0	3	3
15	Total	10	38	2	3	2	5	40	2	3		6	50	2	3	1	4	48	0	0	5	5
	Total all ages	289	1,010	54	63	49	112	1,026	100	109	57	166	1,236	46	56	64	120	1,246	66	75	72	147

[1] Number of noncarious teeth present at the time of the first examination, April 1942.
[2] Number of noncarious teeth that became carious during the year ended May 1943.
[3] Number of surfaces attacked by caries in new carious teeth.
[4] Number of new carious surfaces in teeth that had one or more carious surfaces at the time of the first examination.
[5] The sum of the items in [3] and [4].

all ages in table 2 that 54 teeth became carious in the upper left or treated quadrant and 100 in the upper right or untreated quadrant. This represents a difference of 46.0 percent less new carious teeth in the treated than in the untreated upper teeth. On the basis of percentage of teeth attacked, the rate is 5.3 for the treated and 9.7 for the untreated teeth. The probability that this or a greater difference might occur by chance is 0.0002.

In the lower jaw, 46 new carious teeth occurred in the left and 66 in the right quadrant. This is a difference of 30.3 percent less carious teeth in the treated than in the untreated teeth. The percentage of teeth attacked is 3.7 for the treated and 5.3 for the untreated lower teeth. The probability that this or a greater difference might occur by chance is 0.0574. Although this difference is not statistically significant, the fact that in eight of the nine age groups fewer teeth became carious in the treated than in the untreated quadrant and the fact that the difference is in accord with the findings in the upper teeth strongly suggest that the observed difference is real but that the available experience is insufficient to be conclusive.

The total number of new carious teeth in both treated quadrants is 100 and that for both untreated quadrants is 166. This is a gross difference of 39.8 percent less teeth attacked by caries in the treated than in the untreated teeth. In terms of percentage caries attack rates for the previously noncarious teeth, 4.4 percent of the treated and 7.3 percent of the untreated became carious during the study year.

Since the number of tooth surfaces attacked by caries is highly correlated with the number of teeth attacked, it is to be expected that the difference in the number of new carious tooth surfaces occurring in the teeth of the treated and untreated quadrants of the mouth would be similar to the differences indicated in terms of numbers of teeth attacked. The direction of the difference is the same—112 and 166 new carious tooth surfaces in the treated and untreated upper quadrants, respectively, and 120 and 147 in the treated and untreated lower quadrants, respectively. In the upper quadrants, there were 32.5 percent less and in the lower quadrants there were 18.4 percent less new carious tooth surfaces in the treated than in the untreated teeth. For all teeth, the gross difference between the number of new carious tooth surfaces in treated and untreated teeth is 25.9 percent. However, the order of this difference is appreciably less than when the comparison was made on the basis of teeth instead of tooth surfaces. An explanation for this finding can be obtained from a comparison of the number of new carious tooth surfaces in previously attacked teeth. It will be noted that although the totals are less for the treated than the untreated—49 in the treated and 57 in the untreated upper teeth, and 64 in the treated and 72 in the untreated lower teeth—the differences are relatively slight. Furthermore, when the comparison is made for each age group, it will be found that the number of instances in which the new carious tooth surfaces are fewer in the treated than in the untreated previously attacked teeth is markedly similar to the number of instances in which the opposite result is obtained. The conclusion follows that either the treatment is not effective in preventing caries attack on surfaces of previously attacked teeth or the order of its effectiveness is not great enough to be determined from these data.

TABLE 4.—*The number, and the average number of decayed, missing, or filled (DMF) permanent teeth per child for 289 treated children and 326 control children, by age, April 1942*

	Age								
	7	8	9	10	11	12	13	14	15
	Number of DMF teeth								
Treated group	18	42	75	178	232	340	309	517	104
Control group	14	22	47	81	86	300	459	784	487
	Average number of DMF teeth per child								
Treated group	1.80	2.33	2.88	4.34	5.95	6.67	7.02	10.34	10.40
Control group	1.08	2.20	2.76	4.50	5.73	6.98	7.06	8.34	9.55

TABLE 3.—*Number of children in the treated group according to number of topical applications of sodium fluoride solution to the teeth in the left quadrants of the mouth, and according to school attended*

School	Number of treatments									
	7	8	9	10	11	12	13	14	15	Total
North Mankato	....	....	....	....	1	1	4	15	85	106
Arlington	....	1	....	....	....	4	12	13	70	100
St. Louis Park	5	78	....	....	....	....	....	....	....	83
All schools	5	79	....	....	1	5	16	28	155	289

The foregoing analysis of findings in the treated groups has been made without differentiation according to number of treatments given. The distribution of the children with respect to the number of treatments received is presented in table 3.

Since all but 51 of the North Mankato and Arlington children received the maximum number of 15 treatments and all but 5 of the St. Louis Park children received the maximum number of 8 treatments, a comparison of the findings within these two groups with respect to number of treatments is not indicated. However, a comparison of the caries experience in the St. Louis Park children with that in the North Mankato and Arlington children is warranted. An examination of the data on the age specific caries experience presented in table 2 gives no indication that the North Mankato and Arlington children who received roughly twice as many treatments as the St. Louis Park children were benefited by the additional treatments.

During administration of the treatment, complete wetting of the crown surfaces of the central incisors was not obtained in most instances without wetting the mesial surface of the central incisors in the untreated quadrants. The effect of this partial wetting is not known. However, 9 central incisors in the treated quadrants and 13 in the untreated quadrants were attacked by caries. Since the difference in caries experience in the left and right central incisors was similar to that for all teeth in the treated and untreated quadrants, the general results of the analysis would not be influenced by removing these teeth from consideration.

It is not unlikely that topical applications of fluoride to the teeth in two quadrants of the mouth might have a general effect on the environment and caries experience of the teeth in the un-

TABLE 5.—*Control group. Dental caries experience during year ended May 1943, in the permanent teeth of 326 Minnesota children*

Age	Urban center	Number of children	Upper left: P teeth[1]	Upper left: New DF teeth[2]	Upper left: DF surfaces in new DF teeth[3]	Upper left: New DF surfaces in DF teeth[4]	Upper left: Total new DF surfaces[5]	Upper right: P teeth[1]	Upper right: New DF teeth[2]	Upper right: DF surfaces in new DF teeth[3]	Upper right: New DF surfaces in DF teeth[4]	Upper right: Total new DF surfaces[5]	Lower left: P teeth[1]	Lower left: New DF teeth[2]	Lower left: DF surfaces in new DF teeth[3]	Lower left: New DF surfaces in DF teeth[4]	Lower left: Total new DF surfaces[5]	Lower right: P teeth[1]	Lower right: New DF teeth[2]	Lower right: DF surfaces in new DF teeth[3]	Lower right: New DF surfaces in DF teeth[4]	Lower right: Total new DF surfaces[5]
7	North Mankato	13	23	5	5	0	5	21	2	2	0	2	25	1	2	1	3	27	1	1	2	3
8	North Mankato	10	22	0	0	1	1	21	1	1	2	3	26	1	1	1	2	24	1	1	2	3
9	North Mankato	7	19	3	3	2	5	16	1	1	0	1	21	0	0	0	0	19	1	1	0	1
9	Arlington	10	24	1	1	2	3	22	0	0	1	1	26	1	1	0	1	30	1	2	3	5
9	Total	17	43	4	4	4	8	38	1	1	1	2	47	1	1	0	1	49	2	3	3	6
10	North Mankato	6	16	1	1	2	3	20	2	2	1	3	29	1	1	0	1	24	2	3	2	5
10	Arlington	12	36	11	13	3	16	34	10	11	4	15	34	2	2	1	3	35	1	1	7	8
10	Total	18	52	12	14	5	19	54	12	13	5	18	63	3	3	1	4	59	3	4	9	13
11	North Mankato	11	27	0	0	6	6	30	3	3	2	5	46	0	0	1	1	49	1	2	4	6
11	Arlington	4	20	1	1	0	1	19	0	0	0	0	20	0	0	1	1	19	1	1	1	2
11	Total	15	47	1	1	6	7	49	3	3	2	5	66	0	0	2	2	68	2	3	5	8
12	North Mankato	5	24	2	2	0	2	24	1	1	0	1	22	0	0	3	3	24	2	3	2	5
12	Arlington	5	26	0	0	3	3	25	3	3	0	3	27	1	2	1	3	28	3	3	3	6
12	St. Louis Park	33	155	18	21	6	27	153	16	18	3	21	177	10	11	8	19	175	9	11	7	18
12	Total	43	205	20	23	9	32	202	20	22	3	25	226	11	13	12	25	227	14	17	12	29
13	Arlington	6	34	3	4	3	7	33	5	6	1	7	35	4	5	1	6	33	0	0	4	4
13	St. Louis Park	59	263	30	34	30	64	265	38	45	21	66	293	18	23	26	49	296	16	17	25	42
13	Total	65	297	33	38	33	71	298	43	51	22	73	328	22	28	27	55	329	16	17	29	46
14	Arlington	12	57	3	4	1	5	59	5	7	6	13	67	1	1	3	4	64	4	5	4	9
14	St. Louis Park	82	364	31	38	33	71	364	35	39	46	85	404	22	25	28	53	420	28	34	24	58
14	Total	94	421	34	42	34	76	423	40	46	52	98	471	23	26	31	57	484	32	39	28	67
15	Arlington	20	91	10	10	5	15	86	4	3	5	8	98	4	5	9	14	96	6	7	4	11
15	St. Louis Park	31	131	9	11	17	28	128	12	16	22	38	146	7	10	5	15	146	5	8	17	25
15	Total	51	222	19	21	22	43	214	16	19	27	46	244	11	15	14	29	242	11	15	21	36
	Total all ages	326	1,332	128	148	114	262	1,320	138	158	114	272	1,496	73	89	89	178	1,509	82	100	111	211

[1] Number of noncarious teeth present at the time of the first examination, April 1942.
[2] Number of noncarious teeth that became carious during the year ended May 1943.
[3] Number of surfaces attacked by caries in new carious teeth.
[4] Number of new carious surfaces in teeth that had one or more carious surfaces at the time of the first examination.
[5] The sum of the items in [3] and [4].

treated quadrants. In order to measure this effect, a negative control group of children was studied concurrently with the treated group. The only means of determining the comparability of the expected new caries experience in these two groups of children for the year ended May 1943 is to ascertain the similarity or dissimilarity in their past caries experience. Data on the prevalence of dental caries in the treated and control groups of children at the time of the first examinations are presented in table 4.

The age specific prevalence of dental caries in the treated group and that in the control group (table 4) are quite similar. The numerical average of the nine age specific rates for the treated and for the control group are 5.75 and 5.36, respectively. Because of this similarity, it seems reasonable to expect that for the year following the first examination the incidence of dental caries in the treated group would normally be approximately the same as that in the control group.

Data on the incidence of dental caries in the control group are presented in table 5. From the totals for all ages, it will be noted that in the upper jaw there are 10 more new carious teeth in the right than in the left quadrant, and in the lower jaw there are 9 more in the right than in the left quadrant. The left quadrants had 8.6 percent less new carious teeth than the right quadrants. This digression from bilateral symmetry in the occurrence of dental caries suggests the possibility of a slight systematic error. However, the difference is not statistically significant. Furthermore, a comparison of the number of new carious teeth in the upper left quadrant with the number in the upper right quadrant, for each of the nine age groups, results in the finding that 4 are less, 3 are greater, and 2 are equal. When similar comparison is made between the findings in the lower left and lower right quadrants, the results are 4 less, 1 greater, and 4 equal. This ordering of the differences by age indicates that the differences observed in the totals were due to chance.

In order to facilitate a direct comparison of the new caries experience in the treated and control groups, a part of the data given in tables 2 and 4 is rearranged and presented in table 6, to-

TABLE 6.—*Number and percentage of previously noncarious permanent teeth attacked by caries during the year ended May 1943, by age, and left and right mouth quadrants, for the treated and control children*

Age	Treated group: Left quadrants: P teeth[1]	Treated group: Left quadrants: New DF teeth[2]	Treated group: Left quadrants: Percent DF[3]	Treated group: Right quadrants: P teeth[1]	Treated group: Right quadrants: New DF teeth[2]	Treated group: Right quadrants: Percent DF[3]	Control group: Left quadrants: P teeth[1]	Control group: Left quadrants: New DF teeth[2]	Control group: Left quadrants: Percent DF[3]	Control group: Right quadrants: P teeth[1]	Control group: Right quadrants: New DF teeth[2]	Control group: Right quadrants: Percent DF[3]
7	36	1	2.8	37	2	5.4	48	6	12.5	48	3	6.2
8	67	5	7.5	75	7	9.3	48	1	2.1	45	2	4.4
9	157	6	3.8	163	13	3.0	90	5	5.6	87	3	3.4
10	252	10	4.0	261	19	7.3	115	15	13.0	113	15	13.3
11	312	14	4.5	317	16	5.0	113	1	.9	117	5	4.3
12	469	16	3.4	459	35	7.6	431	31	7.2	429	34	7.9
13	429	23	5.4	442	42	9.5	625	55	8.8	627	59	9.4
14	435	21	4.8	430	30	7.0	892	57	6.4	907	72	7.9
15	88	4	4.5	88	2	2.3	466	30	6.4	456	27	5.9
Total	2,245	100	4.4	2,272	166	7.3	2,828	201	7.1	2,829	220	7.8
Average rate[4]			4.5			6.8			7.0			7.0

[1] Noncarious (without caries or fillings) at time of first examination, April 1942.
[2] Noncarious teeth [1] attacked by caries during the year ended May 1943.
[3] Percent of noncarious teeth [1] attacked by caries during year ended May 1, 1943.
[4] Numerical average of the nine age specific rates.

gether with age specific percentage attack rates.

This arrangement of the data (table 6) makes available another type of comparison between the new caries experience in the treated and untreated mouth quadrants of the treated group. However, the specific purpose in presenting this arrangement is to afford direct comparisons between the caries experience in the right or untreated quadrant of the treated group and that in the same quadrants of the control group. Since the age distribution of the children in the treated and control groups is dissimilar, an additional summary figure is presented, the average rate. This figure is merely the numerical average of the age specific rates, and thus gives equal weight to each of the nine age specific rates.

It will be noted (table 6) that the average rate for the percentage of teeth attacked by caries is 6.8 for the right or untreated quadrants of the treated group and 7.0 for the right quadrants of the control group. Further evidence of marked similarity in the new caries experience in the right quadrants of the treated and untreated groups is obtained from comparison of the age specific percentage attack rates. Although there is considerable variation in these age specific rates, the differences are equally divided, 4 being higher and 5 being lower in the treated than in the control group. Inasmuch as dental caries normally occurs in bilaterally equal distribution, it is not necessary to limit the comparison to the right quadrant of the control group. The result obtained when the average rate for the right quadrants of the treated group is compared with the average rate for the left quadrant of the control group is again 6.8 and 7.0.

The striking similarity in the new caries experience in the right or untreated mouth quadrants of the treated group and that in the right (or left) quadrants in the control group indicates that the effect of topical applications of fluoride is exclusively local during the first year after application. This is evidence that the teeth in the treated and the teeth in the untreated mouth quadrants of the treated group actually retain the intended relationship of experimental and control for the period of experience presented in this report.

DISCUSSION

The results of this analysis demonstrate that topical application of a 2-percent sodium fluoride solution to the teeth under the conditions of this investigation was effective in reducing the incidence of dental caries by roughly 40 percent. However, the evidence applies only to that effect produced during the first year following the applications and to a group of children with an average incidence of approximately one DMF permanent tooth per year. The duration of the effect is not yet known.

Apparently the treatment is not effective in preventing caries attack on the noncarious surfaces of teeth previously attacked. If this finding is substantiated by continued studies, it has important implications which may assist in determining the physical or chemical action of fluorides on enamel. Further, it would indicate that the effectiveness of the treatment is largely limited to the prevention of caries and that it is not effective in controlling active dental caries. Therefore, the validity of this finding will also be an important factor in establishing the procedures to be employed in conducting a program of topical applications of fluoride to the teeth.

Since the criterion for diagnosing arrested caries is highly subjective, in the judgment of the examiner there was no evidence that the treatment effected an arrest of the active caries. However, this opinion is in accord with the findings on new carious surfaces in teeth previously attacked by caries. The question of whether or not the treatment arrests active caries is important to the interpretation of these findings. If arrest is effected, then these findings probably represent the true difference in caries experience in the treated and untreated teeth. On the other hand, if arrest is not effected, then these findings represent a conservative estimate of the difference. This is true because undoubtedly some of the new caries in the treated teeth was initiated prior to treatment but was not detected during the first examination.

As stated previously, it was not the intent of this study to determine the minimum number of treatments necessary to obtain the full effectiveness of the fluorides. However, the findings do indicate that 15 treatments were not more effective than 8 treatments. It is possible that one thorough treatment would be fully as effective as multiple treatments, but the answer to this question remains to be determined by future studies.

Although a 2 percent solution of sodium fluoride was used in this study, the most effective concentration of this solution or of other fluorides is not known. A 2 percent solution of sodium fluoride is highly toxic and must be used and guarded with extreme caution. Certainly the optimum concentration of the fluoride solution would be the minimum effective concentration.

SUMMARY

Data on the incidence of dental caries in the permanent teeth of 289 treated children and 326 control children for the year ended May 1943 have been presented and analyzed. During the first 8-week period of the observation the children in the treated group received 7 to 15 topical applications of sodium fluoride solution to the teeth in the left quadrants of the mouth.

Analysis of the data indicates that during the year of these observations:

1. Approximately 40 percent less new carious teeth occurred in the treated than in the untreated teeth of the treated group of children.
2. The relative reduction in the incidence of dental caries in treated teeth was appreciably greater in the upper than in the lower teeth.
3. There was no significant difference between the number of new carious tooth surfaces in treated and untreated teeth which had been previously attacked by caries. This finding indicates that the treatment was not effective in inhibiting renewed attacks by caries on teeth already attacked.
4. The incidence of dental caries in teeth in the untreated mouth quadrants of the treated group was similar to that in teeth in the comparable mouth quadrants of the control group. This finding indicates that for the first year following treatment the caries inhibiting effect is local and limited to those teeth to which the fluoride is topically applied.

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THE EFFECT ON CARIES INCIDENCE OF A SINGLE TOPICAL APPLICATION OF A FLUORIDE SOLUTION TO THE TEETH OF YOUNG ADULT MALES OF A MILITARY POPULATION

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(All clinical examinations were made by E. M. Short)

Epidemiological evidence shows that individuals using, throughout life, domestic waters containing small amounts of fluoride experience a relatively low amount of dental caries. Some of the epidemiological aspects of the fluorine-dental caries relationship have been recently reviewed by Dean (*1*).

In order to test further the inhibitory effects of fluorides on dental caries other avenues of approach are being investigated. Results of studies designed to determine whether or not fluoride solutions applied to the external surfaces of the teeth would decrease their susceptibility to caries attack have been reported (*2*, *3*, *4*). While these studies are still in the experimental stage, the results have suggested a beneficial effect. The three studies reported were made on children and multiple applications of fluoride solutions were used during the study period.

The primary purpose of this study was to learn whether or not a single application of a fluoride solution would appreciably lower the incidence of dental caries in young male adults living under military conditions. If multiple applications were necessary to decrease the caries incidence, the value of this prophylaxis would be largely nullified insofar as its use in the armed forces is concerned. Also as a public health procedure the value of topical application of fluorides to control dental caries obviously decreases as the number of treatments increases. The present study was made at the U.S. Coast Guard Academy, New London, Connecticut. All regular cadets of the 2d and 3d classes were included in the study and the period of observation was one year. All cadets ate in the regular Academy cadet mess.

METHODS

In September 1942, all cadets of the 2d and 3d classes were given a complete dental examination by a dental officer using the facilities of the Academy dental clinic. Bite-wing radiograms were taken of each individual, using four posterior and three anterior films. Each cadet filled out a questionnaire showing his place of residence during the first 8 years of life, indicating whether urban or rural. These records were studied and classified as to whether or not the individual was likely to have used fluoride-containing waters during the formative period of his teeth. The results of this classification were recorded on his examination schedule together with the clinical observations regarding the presence or absence of dental fluorosis. At the time of the first examination, twice during, and once at the end of the study year, saliva samples were collected from each cadet and *L. acidophilus* counts determined. All counts were made by one of the authors (FAA) and all saliva specimens were collected in the morning prior to brushing of the teeth and eating.

After completing the clinical and bacteriological examinations, the record of each individual was matched with that of another, one record being put into group A (control) and the other into group B (experimental). In order to make "matched pairs" of these records the following criteria were given consideration; (a) the number of *L. acidophilus* found present in the saliva, (b) the presence or absence of suggested fluoride exposure during the first 8 years of life as indicated by the clinical examination or by residence history, (c) the past caries experience of the individual taking into account the number of teeth showing fillings, extractions, or untreated lesions, and giving consideration to the extent and location of this caries experience, (d) the age of the individual and his class in the Academy, i.e., 2d or 3d. During the process of matching the records the order of precedence of these various criteria was the same as given above. Thus each cadet in group A had a partner in group B who had what might be called a "comparable oral condition."

In order to eliminate the chance of personal bias the dental officer (D. E. S.) applying the fluoride treatments had no knowledge which solution contained fluorides. He was given two solutions (one for spray, the other for topical application) for each group of cadets. The composition of the different solutions was as follows:

1. Solutions for topical application—

   Solution A (control solution containing sodium chloride):

Sodium chloride	0.26 molar
Acetic acid	1.50 percent
Sodium hydroxide	0.21 percent

   This solution had a pH of 4.0 at time of preparation; at completion of treatments the pH was 4.5.

   Solution B (5000 ppm. fluorine as sodium fluoride):

Sodium fluoride	0.26 molar
Acetic acid	1.50 percent

   This solution had a pH of 4.0 at time of preparation; at completion of treatments the pH was 4.6.

2. Solutions used in spray bottles—

   Solution A (control solution of sodium chloride):

Sodium chloride	0.0526 molar

   Solution B (1000 ppm. fluorine as sodium fluoride):

Sodium fluoride	0.0526 molar

The method of applying the above solutions to the teeth was as follows: A complete prophylaxis was given immediately prior to and at the same sitting as the application of the solutions. The prophylaxis consisted of thoroughly scaling the teeth and polishing with pumice which had been mixed with a small amount of the treatment solution. Rubber cups were used on all surfaces of the teeth except the occlusal which were cleaned with brushes. A spray solution, as described above, was used to irrigate the mouth during the prophylaxis and at no time was water used for rinsing. Dental floss was used to clean and work the solution into contact with the proximal surfaces. After the prophylaxis separate quadrants of the mouth were isolated with cotton rolls and the area thoroughly dried with warm air. The treatment solution was then applied liberally with cotton pellets and dried onto the teeth with warm air. This procedure was repeated three times on each quadrant in a period of 5 minutes. No other fluoride treatment was given to these cadets during the study year, however, they received routine dental treatment if they needed it. No additional prophylaxes were given during the one year study period.

In January and July of the study year, saliva samples were collected and *L. acidophilus* counts determined as at the start of the study. In September 1943, a second complete dental examination was made by the same examiner using the same equipment as was used at the start of the study. The examiner had no contact with the study except to make the initial and final examinations and the latter was made without reference to the findings of the first examination. The second examination, like the first, was supplemented by the use of bite-wing radiographs, and saliva samples were collected and *L. acidophilus* counts determined.

RESULTS

At the beginning of this study there were 258 cadets in the 2d and 3d classes at the Academy, and their mean age was 19.6 years. The specific ages in September 1942, according to last birthday, were as follows: 16—17 years old, 41—18 years old, 62—19 years old, 68—20 years old, 45—21 years old, 25—22 years old, 1—23 years old. During the study year a number of cadets resigned from the Academy, mostly for scholastic reasons. In September 1943 the study group consisted of only 211 cadets. In this number there remained only 94 "matched pairs." There were 23 cadets in the Academy who were examined both years but whose partners were lost to the study, therefore the findings on these 23 cadets are not included in the comparisons. Of the 47 cadets who dropped out of the study 31 were from group A and 16 were from group B. It is interesting to note that such a difference in the numbers dropping out of each group would occur less than three times in 100 trials by chance alone. All comparisons of results between 1942 and 1943 were made on this latter group. In table I are given the results in respect to caries experience as observed in the 1942 clinical examination of the 258 cadets; also the same observations on the 188 cadets who remained throughout the study as "matched pairs" and constitute the basis of this report. It can be seen from these results that the two groups of cadets who remained in the study throughout the year are representative of the original group.

It may be noted that the results shown in table I are from the clinical examination alone and are meant only to give a general picture of the dental conditions as they were at the start of the study. In order to more accurately measure the gain in carious teeth or surfaces the radiographic findings were added to the above results. At the same time obvious errors in the clinical examination were edited so that the caries incidence could be determined for each group using the "edited" results for 1942 as a base line and similar results for 1943 for comparison. The editing consisted of correcting the schedule form to take care of obvious errors such as having a tooth show a filling in 1942 and none in 1943 while the radiograph indicated a filling present both years; or the opposite. Also, a tooth which was marked as carious in 1942 was considered carious in 1943 regardless of whether the clinical examination for 1943 had it listed as carious except in those cases where the radiograph showed the opposite to be true. These editing changes were equally distributed in both groups. An idea of the minor influence of these changes can be gained by comparing the total caries experience of the two groups in 1942 as shown in tables I and II. In table II are given the results of the two examinations both in respect to the number of teeth showing caries experience either by clinical or by clinical plus radiograph examinations and in

Table I.—*Showing the similarity in 1942 of the dental caries experience in the 258 cadets originally examined and the 188 cadets of this group who remained throughout the period of observation*

	No. examined	Filled teeth (past dental caries)	Teeth with untreated caries	Missing teeth	Total caries experience
Number					
Original group	258	2230	217	125*	2573
Group A (control)	94	823	79	46*	948
Group B (experimental)	94	813	89	47	949
Number per 100 Cadets					
Original group		864	84	48	997
Group A (control)		876	84	49	1009
Group B (experimental)		865	95	50	1010

* Includes one tooth diagnosed as "extraction indicated."

Table II.—*Results of clinical and radiographic examinations 1 year apart of the 94 "matched" pairs of cadets in the control (A) and experimental (B) groups*

	1942*		1943*		Increase	
	Group A	Group B	Group A	Group B	Group A	Group B
No. teeth showing caries experience (clinical exam.)	955	957	1013	1021	58	64
No. teeth showing caries experience (clinical & radiographic	1078	1071	1133	1130	55	59
No. carious surfaces or areas (clinical exam.)	1943	1956	2149	2169	206	213
No. carious surfaces or areas (clinical & radiographic	2295	2274	2500	2446	205	172

* Both groups contain 94 persons.

respect to the caries index. The caries index for each individual was calculated according to the method described by Bodecker (*6*). It is, essentially, a method to evaluate the number of tooth surfaces or areas attacked by caries.

It has been shown by a number of workers, the Michigan group in particular, that whenever there is a change in the dental caries incidence of individuals the change is associated with an alteration in the *L. acidophilus* count of the saliva (*5*).

Therefore, if a change has occurred in the dental caries incidence in group B as compared with group A, which has not been elucidated by the clinical and radiographic examinations, a comparison of the *L. acidophilus* counts obtained during the year might show such a change. The results of these counts as shown in table III give no indication of any difference in the caries attack rate between the control and treated groups.

Table III.—*Numerical distribution of oral L. acidophilus counts on the salivas of the 188 cadets in the control (A) and experimental (B) groups*

Number lactobacilli per cc. of saliva	Sept. '42		Jan. '43		June '43		Sept. '43	
	A.	B.	A.	B.	A.	B.	A.	B.
Negative and <100	18	16	19	19	21	21	14	12
100 to <1000	8	11	8	8	18	13	7	4
1000 to <10,000	21	21	14	20	13	19	13	9
10,000 to <20,000	4	8	4	7	7	12	9	11
20,000 to <40,000	12	10	17	10	11	7	7	13
40,000 to <80,000	12	19	13	17	7	12	16	17
80,000 and over	19	9	19	12	14	7	28	28
Total examined	94	94	94	93	91	91	94	94

DISCUSSION

As stated previously, this study was planned to determine whether a significant reduction in caries incidence in adults of military age could be brought about by a single application of a fluoride solution. The findings of this study, as shown in tables II and III, indicate no reduction in caries incidence in the fluoride-treated group (B) either by clinical, radiographic or bacteriological examinations.

Although the results of this experiment are negative in so far as any beneficial effect on dental caries control is concerned, some of the findings deserve further discussion. The method used in this study for selecting the experimental and control groups differs from the usual methods employed. Occasionally when experiments are planned to determine the effect of caries control measures, attempts are made to have the experimental and control groups comparable as to age, sex, and number of teeth showing dental caries experience. Little, if any, attention is paid to factors such as the conditions under which the teeth were formed or the oral environment at the time of the study. Yet it is generally agreed that these conditions do play a role in the etiology of the disease. An attempt has been made in the present study to equalize the known variables by the method used in matching or pairing the experimental and control groups.

The importance of one of these variables can be judged by considering the result of comparing the caries incidence in those individuals who had high *L. acidophilus* counts at the start of the study with those who had low or negative counts. The clinical and radiographic examinations show that the 59 cadets who had high counts (40,000 and over) at the start of the study developed almost 3 times as many new carious surfaces or areas as did the 53 cadets with negative or low (<1000) counts; average 2.8 and 1.1 respectively. Had this factor not been taken into consideration at the start of the study and the two groups balanced accordingly, one group may have had a disportionate number of individuals with high (or low) counts and the results affected accordingly. A similar circumstance would be the conditions associated with the formation of the teeth. An analysis of the questionnaires submitted at the start of the study shows that in the 188 cadets of groups A and B, 15 gave histories indicating residence in known fluoride areas during the first 8 years of life and 44 gave histories suggesting questionable fluoride exposure. A positive diagnosis of dental fluorosis was made clinically in only 3 of these 59 cases, 10 other cases diagnosed as "questionable." It is logical to assume that such a factor would play an important role in any study on caries incidence, especially when the experiment is planned to determine the effect of topically applied fluoride solutions.

In this study the use of bite-wing radiographs to supplement the clinical examinations has permitted certain observations of interest. Although the radiographic results did increase the number of teeth showing caries experience, this increase occurred equally in the two groups. However, in 1943 there were 122 "new" carious teeth found in the 188 cadets by *clinical* examination. Of these "new" carious teeth, 86 had proximal lesions; 79 of these 86 showed evidence of caries by radiograph in 1942. In other words, 79 of the 86 "new" proximal lesions of caries discovered by clinical examination in 1943 were discernible by radiograph in 1942. This finding indicates that without the aid of radiographs clinical examinations 1 year apart may be inadequate to determine whether or not the initiation of enamel caries has been interrupted. This observation may also permit an interpretation of reported results (*3*, *4*) to indicate that topical application of fluorides prevents the progress of caries in the dentin rather than its initiation in the enamel. It should be noted that results reported by McClure (*7*) in regard to the post-eruptive addition of fluorine to the dentin of rats' molar teeth would appear to support such an interpretation.

Attention may be called to the fact that these cadets ate at the regular mess consuming the usual excellent diet of a U.S. Government military academy. They also represent a "selected" sample in regard to dental caries susceptibility since most individuals who are highly susceptible would be barred from entrance to the Academy due to an early loss of teeth. In spite of the foregoing, the results of this study (table II) indicate the cadets developed an average of 0.65 "new" carious teeth per person per year. This incidence of dental caries is similar if not greater than the normal expectancy in general population groups of similar age (*9*). Such results seemingly give little encouragement to the use of so-called "optimum" diets for the control of dental caries in the general population.

SUMMARY

The results of a study in which a single topical application of fluoride was made to the teeth of cadets at the U.S. Coast Guard Academy are reported:

1. After a complete dental examination, including radiographs and *L. acidophilus* counts, the cadets were divided into two groups on a "matched pair" basis. Each member of one group had a partner in the other group who had a comparable oral condition in regard to the following: *L. acidophilus* count, fluoride exposure during the first 8 years of life, past caries experience, and age. Following a prophylaxis a sodium fluoride solution containing 5000 ppm. F, was applied to the teeth of one group (experimental) and a sodium chloride solution of equivalent concentration was applied to the teeth of the cadets in the second group (controls). The period of observation was one year.

2. The observations made by the clinical examinations at the beginning and the end of the study period gave the following results: (a) The cadets of the control and treated groups developed about the same number of new carious teeth, averaging 0.62 and 0.68 respectively; (b) the two groups showed similar gains in the number of new carious surfaces or areas as measured by Bodecker's index—controls averaged 2.2, experimental averaged 2.3.

3. Bacteriological examinations made on the saliva at four different times during the study year gave no indication of any change in the distribution of high and low *L. acidophilus* counts occurring in either of the groups.

4. There is no evidence in this study that a single topical application of a fluoride solution to the teeth of young adults as here carried out would result in reduction of dental caries incidence.

The authors gratefully acknowledge the assistance and cooperation rendered by the officers on duty at the U.S. Coast Guard Academy, New London, Conn.

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THE EFFECT OF TOPICALLY APPLIED SODIUM FLUORIDE ON DENTAL CARIES EXPERIENCE. II. Report of Findings for Second Study Year

By John W. Knutson and Wallace D. Armstrong

In a previous report (*1*) data were presented for the year ending May 1943 on the incidence of dental caries in the permanent teeth of 2 groups of children. The 289 children in the first of these groups received 7 to 15 topical applications of 2 percent sodium fluoride solution to the teeth in the upper left and lower left quadrants of the mouth. The second group, consisting of 326 control children, did not receive the fluoride treatments. Analysis of the data indicated (1) the number of previously undecayed teeth attacked by caries during the study year was approximately 40 percent less in fluoride-treated than in untreated teeth, and (2) the number of additional tooth surfaces attacked in previously decayed teeth was less but not significantly less in treated than in untreated carious teeth.

It is the purpose of this report to present data on the dental caries experience in the permanent teeth of these two groups of children for the second study year and for the 2-year period ending May 1944. Briefly, analysis of the data indicates that during both these time periods initial caries attack on fluoride-treated teeth continued to be approximately 40 percent less than on untreated teeth. Furthermore, the number of additional tooth surfaces attacked in previously decayed teeth not only continued to be less in treated than in untreated carious teeth but the magnitude of the difference was appreciably increased during the second year.

MATERIAL AND METHODS

The children participating in this study comprise a part of the school populations of North Mankato, Arlington, and St. Louis Park, 3 small urban centers in Minnesota. The ages of the children at the time the study was begun varied from 7 to 15 years. The treated group, originally consisting of 337 children, received topical fluoride applications to the teeth in the left quadrants of the mouth. The teeth in the right or untreated mouth quadrants served as direct controls. An additional group of children, originally consisting of 392 children enrolled in the same schools as the treated children, did not receive fluoride treatments and served as additional controls.

During an 8-week period in April and May 1942, each child in the study groups received a dental prophylaxis and a detailed dental examination. In addition the children in the treated group received 7 to 15 topical applications of fluoride to the teeth in the left quadrants of the mouth. The fluoride treatment procedure consisted of isolation of the teeth with cotton rolls, drying the teeth with compressed air, and wetting the crown surfaces of the teeth with 2 percent sodium fluoride solution. The applied solution was allowed to dry in air for approximately 4 minutes. During the 8-week treatment period, roughly two-thirds of the children in the treated group received 2 fluoride applications weekly to a maximum of 15 and the remaining third received 1 application weekly to a maximum of 8 treatments.

The fluoride treatments were completed in May 1942. At yearly intervals thereafter, the teeth of the children in both the treated and control groups have been reexamined. All dental examinations have been made by one of us (J. W. K.). The treated and control children in any one school were randomly examined. Variations in the numbers of children in each study group are due to changes in residence, absence from school at the time reexaminations were conducted or discontinuance of attendance at school. The analysis, throughout, is confined to the dental caries experience in the erupted permanent teeth present at the beginning of the study.

FINDINGS

The caries experience in the permanent teeth of the treated group of children for the 2-year period ending May 1944 is presented, by mouth quadrants, in table 1. Caries experience is expressed in terms of numbers of teeth and tooth surfaces initially attacked during this time period and numbers of additional tooth surfaces attacked in teeth which were carious at the time the first dental examination was made.

According to the data in table 1, only 97 teeth became carious in the upper left or fluoride-treated quadrant whereas 173 teeth became carious in the upper right or untreated quadrant. For the teeth in the lower mouth quadrants, 67 became carious in the left and 107 in the right quadrant. The total number of new carious teeth in both treated quadrants is 164 and for both untreated quadrants is 280. This is a gross difference of 41.4 percent less teeth attacked by caries in the treated than in the untreated teeth and compares closely with the 39.8 percent difference reported (*1*) for the year ending May 1943.

Comparison of the number of tooth surfaces attacked by caries in teeth which were noncarious at the time of treatment (table 1) yields results closely in accord with the results of the foregoing comparisons made on the basis of new carious teeth.

Continuing the examination of the data in table 1, the numbers of tooth surfaces which became carious in previously carious teeth are appreciably less in the treated quadrants than in the

Table 1.—*Treated group. Dental caries experience during the 2-year period ending May 1944, for the permanent teeth in the fluorine-treated and untreated quadrants of the jaws of 270 Minnesota children*

Quadrant	Number of noncarious teeth (April 1942)	New DF[1] teeth (May 1944)	DF surfaces in new DF teeth	New DF surfaces in previously carious teeth	Total new DF surfaces
	Upper				
Treated (left)	929	97	115	94	209
Untreated (right)	940	173	214	120	334
	Lower				
Treated (left)	1145	67	83	89	172
Untreated (right)	1154	107	131	118	249

[1] DF—Carious (decayed or filled).

Table 2.—*Treated group. Dental caries experience during the year ending May 1944, for the permanent teeth in the fluorine-treated and untreated quadrants of the jaws of 260 Minnesota children*

Quadrant	Number of noncarious teeth (May 1943)	New DF[1] teeth (May 1944)	DF surfaces in new DF teeth	New DF surfaces in previously carious teeth	Total new DF surfaces
	Upper				
Treated (left)	842	43	48	70	108
Untreated (right)	816	83	100	86	186
	Lower				
Treated (left)	1,061	27	30	40	70
Untreated (right)	1,055	48	55	61	116

[1] DF—Carious (decayed or filled).

Table 3.—*Treated group. Percent less new caries experience in the fluoride-treated jaw quadrants (left) than in the untreated quadrants (right) of a selected group of Minnesota children*

Year	Upper jaw	Lower jaw	Both jaws
	Percent less new carious teeth		
1942–43	46.0	30.3	39.8
1943–44	48.2	43.8	46.6
1942–44	43.9	37.4	41.4
	Percent less new carious surfaces in previously decayed teeth		
1942–43	14.0	11.1	12.4
1943–44	18.6	34.4	25.2
1942–44	21.7	24.6	23.1

Table 4.—*Control group. Dental caries experience during the 2-year period ending May 1944, for the permanent teeth in the left and right quadrants of the jaws of 320 Minnesota children*

Quadrant	Number of noncarious teeth (April 1942)	New DF[1] teeth (May 1944)	DF surfaces in new DF teeth	New DF surfaces in previously carious teeth	Total new DF surfaces
	Upper				
Left	1,322	253	372	172	501
Right	1,317	254	339	193	532
	Lower				
Left	1,465	137	180	191	371
Right	1,489	137	199	188	387

[1] DF—Carious (decayed or filled).

untreated quadrants—94 in the treated and 120 in the untreated upper teeth, and 89 in the treated and 118 in the untreated lower teeth. These particular findings indicate that the fluoride treatment of carious teeth reduces attack on additional surfaces approximately 20 percent.

In general the findings for the 2-year period of observations are in accord with the findings reported (*1*) for the first year. The only notable difference is an appreciable increase in the difference between the number of additional tooth surfaces attacked in treated as compared with untreated teeth which were carious at the time the study was begun. It is apparent therefore that the caries-inhibiting effect of the topical fluorides is fully as effective during the second year following treatment as during the first year. A direct check on this conclusion is afforded by a separate analysis of the data on the caries experience in the teeth of the treated group for the second year.

The numbers of permanent teeth that were noncarious in May 1943 and the number of these that became carious during the year ending May 1944 are presented for the treated group of children in table 2. The caries experience in terms of tooth surfaces is also given. According to these data, 43 teeth in the upper left or treated quadrant and 83 in the upper right or untreated quadrant were initially attacked by caries during this particular year, a difference of 48.2 percent less caries in the treated than in the untreated upper teeth. In the lower jaw 27 teeth in the left and 48 in the right quadrant became carious, a difference of 43.8 percent. These differences are slightly greater than those observed for either the 2-year period ending May 1944 (table 1) or those observed for the first study year (*1*) and confirm the conclusion that the fluoride treatment is fully as effective during the second year as during the first year following treatment.

A summary of the percentages by which the caries experience in the fluoride-treated teeth was lower than that observed in untreated teeth is presented in table 3 for the separate time periods of the study.

Inasmuch as the control aspect of this study is based on the bilaterally equal occurrence of dental caries usually observed in population groups, it becomes important to check this characteristic in the control group of children. The dental caries experience in the teeth of the control group of children is presented in table 4. Examination of the data in this table shows that 253 teeth in the upper left and 254 in the upper right quadrant were attacked by caries. In the lower jaw, 137 teeth in the left and 137 in the right quadrant became carious. These findings are remarkably in agreement with the observed phenomenon that caries normally occurs bilaterally equal in population groups.

SUMMARY

Data on the incidence of dental caries in the permanent teeth of a treated group of children and a control group of children for the second year of a longitudinal study have been presented and analyzed. The data for the first study year have been reported previously. During an 8-week period, April and May 1942, the children in the treated group received 7 to 15 topical applications of sodium fluoride solution to the teeth in the left quadrants of the mouth. Analysis of the data indicates:

1. During the 2-year period ending May 1944, 41.3 percent less teeth became carious of the fluoride-treated than of the untreated teeth of the treated group of children. The number of additional tooth surfaces which became decayed in teeth which were carious at the beginning of the study was 23.1 percent less in treated than in untreated carious teeth.

2. During the second study year, the year ending May 1944, 46.6 percent less treated teeth became carious than untreated teeth. The number of additional surfaces which became decayed in previously carious teeth was 25.2 percent less in treated than in untreated carious teeth.

3. By and large the findings confirm those reported for the first study year. In addition it is indicated that the fluoride treatments are fully as effective in inhibiting dental caries during the second year following treatment as during the first year. Further, the evidence suggests that the treatment of carious teeth reduces approximately 20 percent the liability to attack on additional surfaces.

4. The incidence of caries in the permanent teeth of the control group of children, by mouth quadrants, was strikingly similar in the two upper quadrants and also in the two lower mouth quadrants.

The foregoing results of this initial or pilot study on the caries-inhibiting effect of topically applied fluorides are sufficiently encouraging to justify additional and more stringently controlled studies. One such study designed to test the relative effectiveness of different numbers of treatments is now in progress and plans for conducting others are being made.

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THE EFFECT OF TOPICALLY APPLIED SODIUM FLUORIDE ON DENTAL CARIES EXPERIENCE. III. Report of Findings for the Third Study Year

By JOHN W. KNUTSON and WALLACE D. ARMSTRONG

In 1942 examinations were made to determine the dental caries experience in the permanent teeth of two groups of Minnesota school children who had been selected for participation in a topical fluoride study. The 337 children in the first of these groups then received 7 to 15 topical applications of 2 percent sodium fluoride solution to the teeth in the upper and lower left quadrants of the mouth. The second group, consisting of 392 control children, did not receive any applications. Subsequent to 1942, annual examinations of the teeth of both groups of children were made and the dental caries experience was recorded. At the end of the first and second study years, the incidence of dental caries in the fluoride-treated as compared with that of the untreated permanent teeth of the treated group of children was reported (*1*, *2*). The incidence of caries in the teeth of treated and control groups was compared also. During the 3-year period since the beginning of the study, the number of children included in the original group who were available for reexamination has declined. At the conclusion of the third study year, only 242 children of the original treated group were available for reexamination.

At the end of the first year it was reported that the number of previously undecayed teeth attacked by caries during that study period was 39.8 percent less in fluoride-treated than in untreated teeth, and the number of additional tooth surfaces attacked in previously decayed teeth was 12.4 percent less in treated than in untreated carious teeth. Analysis of the data at the end of the second year revealed that initial caries attack on fluoride-treated teeth continued to be approximately 40 percent less than on untreated teeth. Moreover, the number of additional tooth surfaces attacked in previously decayed teeth not only continued to be less than in untreated carious teeth, but the magnitude of the difference had increased to 23.1 percent.

It is the purpose of this report to present data on the dental caries experience in the permanent teeth of the treated group of children for the 3-year period ending May 1945, and for the third study year. In summary, the data indicate that the number of teeth initially attacked by caries during the 3-year study period was 36.7 percent less in treated than in untreated teeth. Furthermore, the number of additional tooth surfaces attacked in teeth which were carious at the time of treatment was 23.9 percent less in treated than in untreated carious teeth.

TABLE 1.—*Treated group. Dental caries experience during the 3-year period ending May 1945, for the permanent teeth in the fluoride-treated and untreated quadrants of the mouths of 242 Minnesota children*

Quadrant	*Number of noncarious teeth (April 1942)*	*New DF[1] teeth (May 1945)*	*DF[1] surfaces in new DF teeth*	*New DF[1] surfaces in previously carious teeth*	*Total new DF[1] surfaces*
		UPPER			
Treated (left)	838	130	167	107	274
Untreated (right)	842	205	282	149	431
		LOWER			
Treated (left)	1,032	84	120	109	229
Untreated (right)	1,046	133	182	135	317

[1] DF=carious (decayed or filled).

MATERIAL AND METHODS

Three small urban communities in Minnesota—Arlington, North Mankato and St. Louis Park—provided the school populations from which the children participating in this study were selected. When the study was begun, the children ranged in age from 7 to 15 years. The treated group consisted originally of 337 children, and the control group included 392 children. The former group received applications of sodium fluoride to the teeth in the upper and lower left quadrants of the mouth, the teeth in the upper and lower right quadrants serving as controls. The control group of children did not receive fluoride treatments.

During an 8-week period in April and May 1942, each child in the study groups was given a dental prophylaxis and a detailed dental examination. In addition, the children in the treated group received 7 to 15 topical applications of fluoride to the teeth in the left quadrants of the mouth. The fluoride-treatment procedure consisted of isolating the teeth with cotton rolls, drying the teeth with compressed air, and wetting the crown surfaces of the teeth with a 2 percent sodium fluoride solution. The applied solution was allowed to dry in air for approximately 4 minutes. During the 8-week treatment period, roughly two-thirds of the children in the treated group received two fluoride applications weekly to a maximum of 15, and the remaining one-third received one application weekly to a maximum of eight treatments.

The fluoride treatments were completed in May 1942. The first and all subsequent dental examinations were made at yearly intervals by the same examiner. The treated and control children in any one school were examined at random. The decline which has occurred in the numbers of children in each study group since 1942 has been due to changes in residence, absence from school at the time reexaminations were conducted, or withdrawal from school. Throughout the analysis consideration is confined to the dental caries experience in the erupted permanent teeth present at the beginning of the study. The analysis prepared for the first year is based on 289 children in the treated group and 326 in the control group, and that for the second year is based on 270 treated and 320 control children. The report for the third year is based on 242 treated cases only. Previous data on the control group of children have demonstrated adequately that the occurrence of caries was bilaterally equal and at approximately the same rate as in the untreated teeth of the treated group of children. Therefore, a continuation of reports of findings in the control group does not seem warranted.

FINDINGS

The caries experience in the permanent teeth of the treated group of children for the 3-year period ending May 1945, is presented, by mouth quadrants, in table 1. Caries experience is expressed in terms of numbers of teeth and tooth surfaces initially attacked during this time period and additional tooth surfaces attacked in teeth that were carious in April 1942. The data on caries experience are limited to those permanent teeth present in the mouths in 1942 when the initial dental examinations were made.

According to data presented in table 1, 130 sound teeth became carious in the upper left or fluoride-treated quadrant as compared with 205 in the upper right or untreated quadrant. In the lower mouth quadrants, 84 became carious in the left or treated quadrant and 133 in the right or untreated quadrant. In the treated quadrants the total number of newly carious teeth was 214, as compared with 338 in the untreated quadrants. Thus, there were 36.7 percent less previously sound teeth attacked by caries in the fluoride-treated than in the untreated teeth, during the 3-year period ending May 1945. This difference is only slightly less in magnitude than the difference of 39.8 percent observed 1 year after treatment and the differenc of 41.4 percent noted 2 years following treatment. Comparison of the number of tooth surfaces subsequently attacked by caries, in teeth which were noncarious at the time of treatment, shows results in close accord with the findings reported above. During the 3-year period there were 287 surfaces involved in newly carious teeth in treated mouth quadrants as compared with 464 surfaces in such teeth in untreated quadrants, a difference of 38.1 percent.

The number of additional tooth surfaces attacked by caries in previously carious teeth was also considerably less in treated than in untreated mouth quadronts. In the upper quadrants there were 107 newly carious surfaces in teeth that had been carious at the time of treatment as compared with 149 newly carious tooth surfaces in untreated teeth. In the lower quadrants there were 109 newly carious tooth surfaces in the previously carious teeth of treated quadrants as compared with 135 newly carious surfaces in untreated quadrants. Thus, there were 23.9 percent less newly carious surfaces in previously decayed teeth which had been treated with topical fluoride than in untreated carious teeth.

Data on caries experience for the year ending May 1945 are presented in table 2. These data are concerned only with teeth and tooth surfaces which were noncarious in May 1944 and those attacked by caries during the ensuing year. In the upper left or treated mouth quadrant, 41 teeth became carious during the year as compared with 53 teeth in the upper right or untreated quadrant, a difference of 22.6 percent. In the lower quadrants, 29 teeth became carious during the year on the left or treated side of the mouth, as compared with 37 on the right or untreated side of the mouth, a difference of 21.6 percent. The gross difference for upper and lower quadrants is 22.2 percent. This difference is appreciably lower than the differences ob-

TABLE 2.—*Treated group. Dental caries experience during the year ending May 1945, for the permanent teeth in the fluoride-treated and untreated quadrants of the mouths of 233 Minnesota children*

Quadrant	*Number of noncarious teeth (May 1944)*	*New DF*[1] *teeth (May 1945)*	*DF*[1] *surfaces in new DF teeth*	*New DF*[1] *surfaces in previously carious teeth*	*Total new DF*[1] *surfaces*
		UPPER			
Treated (left)	712	41	48	43	91
Untreated (right)	659	53	74	68	142
		LOWER			
Treated (left)	940	29	39	38	77
Untreated (right)	911	37	45	53	98

[1] DF=carious (decayed or filled).

Table 3.—*Treated group. Percent less new caries experience in the fluoride-treated mouth quadrants (left) than in the untreated quadrants (right) of a selected group of Minnesota children*

Year	Upper jaw	Lower jaw	Both jaws
PERCENT LESS NEWLY CARIOUS TEETH			
1942–43	46.0	30.3	39.8
1942–44	43.9	37.4	41.4
1942–45	36.6	36.8	36.7
1943–44	48.2	43.8	46.6
1944–45	22.6	21.6	22.2
PERCENT LESS NEWLY CARIOUS SURFACES IN PREVIOUSLY CARIOUS TEETH			
1942–43	14.0	11.1	12.4
1942–44	21.7	24.6	23.1
1942–45	28.2	19.3	23.9
1943–44	18.6	34.4	25.2
1944–45	36.8	28.3	33.1

served for the first and for the second study years. However, the actual lessening, if any, of the caries-inhibiting effect of the topical fluoride treatments during the third year of a followup cannot be determined from an analysis of these data. Comparison of the caries experience in the fluoride-treated and untreated teeth by separate years becomes progressively less valid as elapsed time after treatment increases, because of two basic factors. First, the difference in the number of treated and untreated noncarious teeth available for attack becomes greater as time after treatment increases. Second, marked differences in the susceptibility to caries of the several morphological types of teeth, and the early attack of the more susceptible teeth, in greater numbers among untreated than treated teeth, decreases the comparability of the rates at which remaining treated and untreated teeth are attacked. Therefore, the observed differences in caries attack, on fluoride-treated as compared with untreated teeth, become more conservative estimates of the real differences as the observation period increases.

To continue the examination of the data presented in table 2, there were 43 newly carious surfaces in previously decayed teeth in the upper left or treated quadrant as compared with 68 in the upper right or untreated quadrant, and 38 in the lower left or treated quadrant as compared with 53 in the lower right or untreated quadrant. Thus, for the year ending May 1945, as shown in table 3, there were 33.1 percent less newly carious surfaces in previously decayed teeth which were treated than in those which were untreated. This difference is greater than that observed for the first year (12.4 percent) and for the second year (25.2 percent).

SUMMARY

Data on the incidence of dental caries in the permanent teeth of a fluoride-treated group of children, for a 3-year period and for the third year of a longitudinal study, have been presented and analyzed. The data for the first and for the second study years have been presented previously. During an 8-week period, April and May 1942, the children in the treated group received 7 to 15 topical applications of sodium fluoride solution to the teeth in the left quadrants of the mouth. A summary of the data presented here for the first time and of those previously reported indicates:

1. During the 3-year period ending May 1945, the number of permanent teeth initially attacked by caries was 36.7 percent less in fluoride-treated than in untreated teeth. This percentage difference is somewhat smaller than that observed for the first year (39.8) and for the 2-year period ending May 1944 (41.4).
2. During the third study year, or the year ending May 1945, initial caries attack was 22.2 percent less in fluoride-treated than in untreated permanent teeth. This yearly difference is substantially less than that observed for the second year, 46.6, and for the first study year, 39.8.
3. Among permanent teeth which were carious at the beginning of the study in 1942, the number of additional surfaces which became carious during the 3-year period ending May 1945 was 23.9 percent less in treated than in untreated carious teeth. The percentage difference observed for the first study year was 12.4 and for the first 2-year period was 23.1. By individual study years there were 12.4, 25.2, and 33.1 percent less newly carious surfaces in fluoride-treated than in untreated carious teeth, for the first, second and third years, respectively.

ACKNOWLEDGMENTS

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THE EFFECT OF TOPICALLY APPLIED SODIUM FLUORIDE ON DENTAL CARIES EXPERIENCE. IV. Report of Findings With Two, Four, and Six Applications

By JOHN W. KNUTSON, WALLACE D. ARMSTRONG, and FLOYD M. FELDMAN

Reports of studies on the caries-inhibiting effect of fluoride solutions topically applied to the teeth have been made by several investigators. Both the results of such studies and the methods of treatment have varied rather widely. Cheyne (*1*) concluded from his study based on two applications of a 0.05 percent potassium fluoride solution that caries incidence in deciduous teeth was reduced 50 percent by the treatment. Bibby (*3*) reported that a 0.1 percent solution of sodium fluoride applied to the teeth every 4 months effected a 30 percent reduction in caries incidence. Knutson and Armstrong (*4, 5, 6*) applied a 2 percent sodium fluoride solution 7 to 15 times during a 2-month treatment period and obtained a 40 percent reduction. Arnold et al. (7) reported no reduction obtained with one treatment in which 1.0 percent acidulated sodium fluoride was used. Jordan and his associates (*8*) tested the effectiveness of one, two, and three topical applications of 2 percent sodium fluoride solution, and reported 5, 10, and 21 percent reductions, respectively, in caries incidence.

It is evident that variations in results may be due to one or several factors: age of child or more specifically tooth age, type and concentration of the fluoride solution, number of applications in the treatment series, and differences in methods of application. This report presents the results of varying numbers of treatments in which 2 percent sodium fluoride was used throughout as the fluoride solution and in which the applications were not preceded by a dental prophylaxis.

Briefly, the results seem to indicate that omission of prophylaxis prior to initiation of the series of fluoride applications had a noticeable effect. Reduction in caries incidence obtained after two, four, and six topical applications is, on the whole, lower than the reduction obtained in previous studies in which dental prophylaxis was included as part of the treatment procedure. With two fluoride applications, initial caries in fluoride-treated teeth were 9.3 percent less than in untreated teeth. With four and six topical fluoride applications, initial caries in treated teeth was 20.1 and 21.3 percent less, respectively, than in untreated teeth.

MATERIAL AND METHODS

During a 3-month treatment period beginning September 1943, three groups of Rochester, Minn., school children received a series of topical fluoride applications to the teeth in half the mouth. The children in the first of these three groups received two fluoride applications, the second group received four, and the third received six. Half the children in each group were treated in the left side of the mouth and the other half in the right side of the mouth. The teeth in the untreated mouth quadrants served as controls. A dental examination and record of findings was made for each of the 2,016 children participating. The children ranged in age from 7 to 15 years.

The dental examinations were made with mouth mirror and explorer under artificial light and with compressed air available for use at the examiners' discretion. In each case, only the teeth in the upper and lower quadrants of one side the mouth were fluoride-treated. The treatment consisted of isolating the teeth with cotton rolls, drying with compressed air, and wetting the crown surfaces with a 2 percent solution of sodium fluoride. The applied solution was allowed to dry in air for approximately 4 minutes. The series of fluoride treatments was not preceded by and did not include dental prophylaxis. For each child, a maximum of two treatments was given per week, and the treatments were completed in 3 weeks or less.

Two years after the series of fluoride applications, the teeth of the children in the three treatment groups were reexamined. Both the initial and subsequent dental examinations were made by one of us (J.W.K). Although there were initially 2,016 children included in the study, the 2-year report presented here is based on the 1,458 cases available for reexamination. Most of the children not available had moved away, a few had discontinued schooling, and some were absent at the time of reexamination. Analysis of the data on caries experience is confined to the erupted permanent teeth present in the mouth at the time of the initial examination. The age classification of the children refers to age at the time treatment was given.

FINDINGS

The age distribution of the children included in this analysis, distributed by the number of topical applications of sodium fluoride, is shown in table 1. The proportions of children at each age from 7 to 15 included in each of the three treatment groups are approximately equal. Boys and girls are about equally represented, and the children included in each treatment group were selected in about equal proportions from the seven grade schools and two junior high schools in Rochester.

Table 2 presents the caries experience in fluoride-treated and untreated permanent teeth for the 2-year study period by upper and lower mouth quadrants, and separately for the groups of children who had received two, four, and six topical fluoride applications. Table 3 shows the percentage reduction in new caries experience in fluoride-treated teeth, during the 2-year period, compared with untreated teeth.

In the upper jaw quadrants of those children who received two fluoride treatments, 285 fluoride-treated teeth became carious as compared with 316 untreated teeth, a difference of 9.8 percent. In the group that received 4 fluoride treatments, 245 treated teeth became carious as compared with 324 untreated teeth in upper

TABLE 1.—*Age distribution of Rochester, Minn., school children examined at the end of the 2-year study period, showing the number of sodium fluoride applications*

Number of applications	All ages	Children by age at time of treatment								
		7	8	9	10	11	12	13	14	15
2	472	12	61	66	64	68	73	62	53	13
4	504	13	61	76	72	64	72	76	61	9
6	482	14	61	63	72	63	69	66	61	13
Total	1,458	39	183	205	208	195	214	204	175	35

Table 2.—*Dental caries experience during the 2-year period ending November 1945 for permanent teeth in the sodium-fluoride-treated and untreated mouth quadrants of the mouths of 1,458 Rochester, Minn., school children*

Treatment groups by treated and untreated quadrants	Number of noncarious teeth (Sept. 1943)	New DF[1] teeth (Nov. 1945)	DF surfaces in new DF teeth	New DF surfaces in previously carious teeth	Total new DF surfaces
	Upper				
2 applications:					
Treated quadrant	1,692	285	370	165	535
Untreated quadrant	1,684	316	416	213	629
4 applications:					
Treated quadrant	1,818	245	304	178	482
Untreated quadrant	1,810	274	380	197	577
6 applications:					
Treated quadrant	1,726	223	266	160	426
Untreated quadrant	1,740	300	343	208	551
	Lower				
2 applications:					
Treated quadrant	1,939	181	245	186	431
Untreated quadrant	1,920	198	259	206	465
4 applications:					
Treated quadrant	2,053	156	191	170	361
Untreated quadrant	2,039	178	234	188	422
6 applications:					
Treated quadrant	1,964	124	146	176	322
Untreated quadrant	1,947	141	174	224	398

[1] DF=carious (decayed or filled).

mouth quadrants, a difference of 24.4 percent. In the six-treatment group, there were 223 newly carious teeth in upper treated quadrants and 300 in untreated quadrants, a difference of 25.7 percent.

In the lower jaw for the group of children given two fluoride applications, initial caries occurred in 181 treated teeth and in 198 untreated teeth, an 8.6 percent difference. For children who received four fluoride treatments, the number of newly carious teeth in lower quadrants was 156 as compared with 178 in untreated quadrants, a 12.4 percent difference. In the lower jaws of the six-treatment group, 124 treated teeth and 141 untreated teeth became carious, a 12.1 percent difference.

Combining initial caries experience for teeth in upper and lower mouth quadrants, there is an overall difference between treated and untreated teeth of 9.3 percent associated with two fluoride applications, 20.1 percent with four applications, and 21.3 percent with six applications.

Data on the occurrence of newly carious surfaces in previously carious teeth are also presented in tables 2 and 3. The number of additional tooth surfaces which became carious, during the 2-year study period, in teeth which were decayed at the time of treatment was less for fluoride-treated than for untreated carious teeth. The percentage differences were 16.2 for carious teeth given two fluoride applications, 9.6 percent for four applications, and 22.2 percent for six applications. The irregularity in the pattern of these differences is difficult to explain, since it would be expected that four treatments would effect a greater difference than two.

Comparison of the results of this study and that conducted by Jordan and his associates (*8*) is of special interest. In both investigations, 2 percent sodium fluoride solution was topically applied, and the same treatment procedure was used, with the exception that one was preceded by dental prophylaxis and the other was not. In Jordan's study, the reduction in caries incidence associated with one, two and three fluoride applications was 5, 10, and 21 percent, respectively. In the present study, two, four, and six applications effected a 9.3, 20.1, and 21.3 percent reduction, respectively. Thus, four and six applications of 2 percent sodium fluoride solution to the teeth, without prior prophylaxis, were only as effective in inhibiting dental caries as three treatments preceded by a dental prophylaxis. In an earlier study (*4, 5, 6*), teeth treated, after prophylaxis, with 7 to 15 applications of 2 percent sodium fluoride solution had approximately 40 percent less caries than untreated teeth. Analysis of the composite findings of the three studies suggests, therefore, that four fluoride treatments preceded by dental prophylaxis are likely to give the maximum reduction in caries incidence obtainable with 2 percent sodium fluoride solution, using the treatment procedure herein described. Confirmation of this conclusion, however, must await the results of studies now in progress.

SUMMARY

Data on the incidence of dental caries in the permanent teeth of three groups of Rochester, Minn., school children who received two, four, and six applications, respectively, of 2 percent sodium fluoride solution to the teeth in half the mouth have been presented and analyzed. The initial dental examinations and the fluoride treatment series were completed during a 3-month period beginning September 1943, and the followup examinations were made approximately 2 years later in November 1945. The teeth in the untreated half of the mouth served as controls. The treatment procedure used in this study did not include a dental prophylaxis. Analysis of the data indicates that for the 2-year period following the fluoride treatments:

1. The incidence of initial caries in permanent teeth which were noncarious at the time of treatment was 9.3, 20.1, and 21.3 percent less in teeth treated with two, four, and six applications of fluoride solution, respectively, than in untreated teeth.

2. The numbers of additional permanent tooth surfaces which became carious in teeth which were carious at the time of treatment were 16.2, 9.6, and 22.2 percent less in fluoride-treated carious teeth given two, four, and six applications, respectively, than in untreated carious teeth.

Table 3.—*Percentage reduction in new caries experience during the 2-year period ending November 1945 in the permanent teeth of sodium-fluoride-treated mouth quadrants of a group of Rochester, Minn., school children*

Number of applications	Upper jaw	Lower jaw	Both jaws	Number of applications	Upper jaw	Lower jaw	Both jaws
	Percentage reduction in newly carious teeth				Percentage reduction in newly carious surfaces in previously carious teeth		
2	9.8	8.6	9.3	2	22.5	9.7	16.2
4	24.4	12.4	20.1	4	9.6	9.6	9.6
6	25.7	12.1	21.3	6	23.1	21.4	22.2

3. Comparison of the results of this investigation with those previously reported (*4, 5, 6, 8*) indicates that omission of a dental prophylaxis from the treatment procedure materially reduces the caries-inhibiting effects of the topical fluorides.

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THE EFFECT OF TOPICALLY APPLIED FLUORIDES ON DENTAL CARIES EXPERIENCE. V. Report of Findings With Two, Four and Six Applications of Sodium Fluoride and of Lead Fluoride.

By Donald J. Galagan and John W. Knutson

Previous reports (*1, 2, 3, 4*) in this series on the effect of topically applied fluorides on dental caries experience indicated:

1. Approximately 40 percent reduction in dental caries incidence is effected when the initial application of a 2 percent sodium fluoride solution to the teeth is preceded by dental prophylaxis and subsequent applications are made at the rate of one or two per week to a minimum of 7 and a maximum of 15.
2. The caries prophylactic effect of 15 applications is not greater than that obtained with 8 applications.
3. Two, four and six applications of a 2 percent sodium fluoride solution effect 9, 20 and 21 percent reductions, respectively, when the initial application is not preceded by dental prophylaxis.

Jordan and his associates, using a 2 percent sodium fluoride solution and the same technique of application described here, reported 5, 10, and 21 percent reductions in caries incidence respectively following one, two and three applications (*5*). It is the purpose of this report to present the results of additional studies designed to determine the minimum number of applications of 2 percent sodium fluoride required to achieve maximum caries reduction, and to test the comparative effectiveness of lead fluoride. The results of laboratory investigations have suggested that lead fluoride might be more effective than sodium fluoride as a topical agent for the prevention of dental caries (*6, 7*).

Briefly, the results of the study indicate that four applications of a 2 percent solution of sodium fluoride, applied as described in the section on materials and methods, and preceded by dental prophylaxis, are the minimum number required to give maximum reduction in caries incidence. Two applications of sodium fluoride resulted in 21.7 percent less initial caries in treated teeth than in untreated teeth, four treatments reduced initial caries in treated teeth by 40.7 percent, and six resulted in a 41.0-percent reduction. No significant reduction was shown with two, four, or six applications of a saturated lead fluoride solution.

MATERIALS AND METHODS

During a four-month period beginning in January 1946, six groups of Troy and West Milton, Ohio, school children received a series of topical fluoride applications to the teeth in half the mouth. Three of the groups received two, four, and six applications of a 2 percent sodium fluoride solution, respectively. The other three groups received two, four, and six applications of an 0.06 percent lead fluoride solution. Half the children in each group were treated on the right side of the mouth, the other half on the left side. A dental prophylaxis, consisting of necessary scaling and polishing, was administered to each child prior to the initial fluoride treatment.

Table 1.—*Age distribution of 1,555 Miami County, Ohio, school children available for reexamination at the end of a 1-year study period, according to the number of sodium fluoride or lead fluoride applications each received*

Fluoride solution and number of applications	All ages	Children by age at time of treatment								
		7	8	9	10	11	12	13	14	15
Sodium fluoride:										
2 applications	301	33	37	36	41	36	51	32	26	9
4 applications	247	19	20	34	34	36	28	28	28	20
6 applications	259	35	30	26	40	40	31	25	27	5
Total	807	87	87	96	115	112	110	85	81	34
Lead fluoride:										
2 applications	272	31	39	34	33	33	42	24	24	12
4 applications	214	15	19	27	36	32	21	26	28	10
6 applications	262	32	47	32	32	34	21	30	26	8
Total	748	78	105	93	101	99	84	80	78	30

The teeth in the untreated mouth quadrants served as controls. A detailed dental examination was made by one of the authors (D. J. G.) for each of the 1,782 children in the six groups before the first topical application was administered.

The examinations were made with a No. 4 plane mouth mirror and a double end No. 5 explorer under artificial light, and with compressed air available for use at the discretion of the examiner. The treatment consisted of isolating the teeth of the treated side with cotton rolls, drying with compressed air, and wetting the crown surfaces with the fluoride solution. The solution was allowed to dry in air for approximately 4 minutes; then the cotton rolls were removed and the child dismissed. In general, the treatments were given twice a week over a 1-, 2- or 3-week period. All treatments were administered by dental hygienists under the supervision of a dentist.

One year after the series of fluoride applications were completed, the teeth of the children in the six treatment groups were reexamined. At the time of reexamination the examiner had no knowledge as to which teeth had been treated and which were untreated. Of the 1,782 children included in the initial study groups, 1,555 were available for reexamination. Analysis of the data on caries experience is confined to the erupted permanent teeth present in the mouth at the time of the initial examination.

The children ranged in age from 7 to 15 years, and included approximately 90 percent of the children enrolled in elementary and first year high school grades in the public and parochial schools of Troy and West Milton, Ohio. Less than 3 percent of the children were colored. They are distributed throughout the six studies reported, and are included in the analysis. The number of males and females in each age group is approximately equal. The age distribution of the children according to the number of sodium or lead fluoride treatments received, is shown in table 1. Age classification refers to age at the time of the initial examination and treatment.

FINDINGS

Topically applied sodium fluoride.—Table 2 presents the caries experience during the study year in untreated and sodium fluoride treated permanent teeth, by upper and lower mouth quadrants and by number of fluoride applications.

TABLE 2.—*Dental caries experience during the 1-year period ending April 1947, for permanent teeth in the sodium fluoride-treated and untreated mouth quadrants of the mouths of 807 Miami County, Ohio, school children*

Treatment groups by treated and untreated quadrants	Number of noncarious teeth, April 1946	New DF[1] teeth, April 1947	DF surfaces in new DF teeth	New DF surfaces in previously carious teeth	Total new DF surfaces
Upper					
2 applications:					
Treated quadrant	1,058	109	120	78	198
Untreated quadrant	1,082	144	152	72	224
4 applications:					
Treated quadrant	911	70	77	60	137
Untreated quadrant	917	135	148	61	209
6 applications:					
Treated quadrant	857	58	64	45	109
Untreated quadrant	846	89	93	57	150
Lower					
2 applications:					
Treated quadrant	1,174	89	99	86	185
Untreated quadrant	1,168	109	125	94	219
4 applications:					
Treated quadrant	985	70	84	49	133
Untreated quadrant	972	101	112	86	198
6 applications:					
Treated quadrant	967	34	42	47	89
Untreated quadrant	971	67	76	50	126

[1] DF—Carious (decayed or filled).

In the upper jaws of the children who received two applications of sodium fluoride, 109 fluoride-treated teeth became carious, while 144 untreated teeth showed new caries, a difference of 24.3 percent. In the upper mouth quadrants of the 4-treatment group, 70 treated teeth became carious as compared with 135 teeth not treated, a difference of 48.1 percent. In the group that received six treatments there were 58 newly carious teeth in the upper treated quadrants, and 89 in the untreated quadrants, a difference of 34.8 percent.

In the lower mouth quadrants of the children given 2 sodium fluoride applications, 89 newly carious teeth occurred on the treated side and 109 on the untreated side, a difference of 18.3 percent. In the 4-treatment group initial caries occurred in 70 treated lower teeth as compared with 101 untreated teeth, a difference of 30.7 percent. The group receiving 6 applications developed 34 carious teeth in the fluoride-treated lower quadrants and 67 in the untreated quadrants, a 49.3 percent difference.

Combining initial caries experience in upper and lower mouth quadrants, there is a difference between treated and untreated permanent teeth of 21.7 percent achieved by two applications of sodium fluoride, 40.7 percent by four applications and 41.0 percent by 6.

Table 3 shows the percentage less new caries experience in sodium fluoride-treated teeth than in untreated teeth in upper and lower jaws, and in both jaws, during the 1-year period.

Data showing the occurrence of newly carious surfaces in previously carious teeth are also presented in tables 2 and 3. The number of additional surfaces becoming carious was less in the sodium

TABLE 3.—*Percentage less new caries experience during the 1-year perod ending April 1947 in the permanent teeth of sodium fluoride-treated mouth quadrants of a group of Miami County, Ohio, school children*

Number of applications	Upper jaw	Lower jaw	Both jaws
	Percentage less newly carious teeth		
2	24.3	18.3	21.7
4	48.1	30.7	40.7
6	34.8	49.3	41.0
	Percentage less newly carious surfaces in previously carious teeth		
2	−8.3	8.5	1.2
4	1.6	43.0	25.9
6	21.1	6.0	14.0

fluoride-treated than in the untreated carious teeth in all three study groups. There were 1.2 percent less additional carious surfaces in previously carious teeth associated with 2 applications, 25.9 percent with 4 applications, and 14.0 percent with 6.

The difference in the case of the six-treatment group might be expected to be at least as large as that for the four-treatment group, based on the parallel experience of the two groups in numbers of newly carious teeth that developed during the study year. This irregular pattern of difference was noted in the previous study in this series in which prophylaxis was omitted from the treatment procedure (4). The reason for the inconsistency is not known.

Topically applied lead fluoride.—The caries experience in lead fluoride-treated and untreated permanent teeth for the 1-year study period by upper and lower mouth quadrants, and by groups of children who received two, four and six applications is presented in table 4.

Among permanent teeth in upper mouth quadrants, 102 treated teeth in children given 2 applications of lead fluoride as compared with 110 untreated teeth became carious during the study year, a difference of 7.3 percent. In the four-treatment group the same number of newly carious teeth developed in treated as in untreated mouth quadrants, while in the children given 6 lead fluoride applications 76 treated and only 67 untreated permanent teeth decayed, a difference of 13.4 percent in favor of untreated teeth.

In lower mouth quadrants there were 90 new carious treated teeth as compared with 94 untreated teeth among children receiving 2 topical lead fluoride applications, a 4.3 percent difference. In the group receiving 4 topical applications 55 permanent treated teeth became carious as compared with 57 untreated teeth, a difference of 3.5 percent. In the six-treatment group 42 treated and 52 untreated teeth developed initial decay, a difference of 19.2 percent.

Table 5 combines the initial caries experience for the teeth in upper and lower mouth quadrants and in both jaws. Two, four and six applications of lead fluoride resulted in 5.9, 1.6 and 0.8 percent less newly carious teeth, respectively.

Data on the occurrence of newly carious surfaces in previously carious teeth are also presented in tables 4 and 5. The groups receiving two, four and six applications of lead fluoride showed, respectively, a 0.7-percent increase, and 12.2 and 11.5 percent reductions in the number of additional carious surfaces in teeth that were previously carious.

The findings of this study indicate that a 0.06 percent solution of lead fluoride, topically applied as described, is not effective in inhibiting dental caries.

Table 4.—*Dental caries experience during the 1-year period ending April 1947 for permanent teeth in the lead fluoride-treated and untreated mouth quadrants of the mouths of 748 Miami County, Ohio, school children*

Treatment groups by treated and untreated quadrants	Number of noncarious teeth, April 1946	New DF[1] teeth, April 1947	DF surfaces in new DF teeth	New DF surfaces in previously carious teeth	Total new DF surfaces
Upper					
2 applications:					
Treated quadrant	975	102	116	63	179
Untreated quadrant	972	110	120	60	180
4 applications:					
Treated quadrant	754	70	79	59	138
Untreated quadrant	785	70	75	71	146
6 applications:					
Treated quadrant	841	76	85	51	136
Untreated quadrant	828	67	73	59	132
Lower					
2 applications:					
Treated quadrant	1,075	90	100	80	180
Untreated quadrant	1,079	94	106	82	188
4 applications:					
Treated quadrant	868	55	62	63	125
Untreated quadrant	858	57	68	68	136
6 applications:					
Treated quadrant	959	42	49	58	107
Untreated quadrant	960	52	56	63	119

[1] DF—carious (decayed or filled).

SUMMARY

A study of the incidence of dental caries in six groups of children in Miami County, Ohio, in which three of the groups received two, four and six topical applications of a 2 percent solution of sodium fluoride and three groups received two, four and six topical applications of a 0.06 percent lead fluoride solution to the teeth in half the mouth has been presented and analysed. The fluoride treatment series was preceded by a dental examination and prophylaxis and applications were administered at the rate of two per week. The followup examinations were made 1 year after completion of the fluoride treatments. The teeth in the untreated upper and lower mouth quadrants served as controls.

Analysis of the data for the first study year indicate:

1. The incidence of initial caries in permanent teeth noncarious at the time of treatment with two, four and six topical applications of sodium fluoride, was 21.7, 40.7 and 41.0 percent less, respectively, in treated than in untreated teeth.

2. The number of additional permanent tooth surfaces which became carious in previously carious teeth during the 1 year period was 1.2, 25.9 and 14.0 percent less in sodium fluoride treated carious teeth, given two, four and six applica-

Table 5.—*Percentage less new caries experience during the 1-year period ending April 1947 in the permanent teeth of lead fluoride-treated mouth quadrants of a group of Miami County, Ohio, school children*

Number of applications	Upper jaw	Lower jaw	Both jaws
	Percentage less newly carious teeth		
2	7.3	4.3	5.9
4	0.0	3.5	1.6
6	−13.4	19.2	0.8
	Percentage less new carious surfaces in previously carious teeth		
2	−5.0	2.4	−0.7
4	16.9	7.4	12.2
6	15.3	7.9	11.5

tions, respectively, than in untreated teeth.

3. The results of this investigation together with those of studies previously reported indicate that four topical applications of sodium fluoride preceded by dental prophylaxis affords maximum reduction in dental caries incidence (approximately 40 percent).

4. Two, four and six topical applications of a 0.06 percent solution of lead fluoride are not associated with a significant reduction in the incidence of dental caries.

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EFFECT OF TOPICALLY APPLIED FLUORIDE ON DENTAL CARIES EXPERIENCE. VI. Experiments With Sodium Fluoride and Calcium Chloride . . . Widely Spaced Applications . . . Use of Different Solution Concentrations.

By Donald J. Galagan and John W. Knutson

Previous reports in this series (*1, 2, 3, 4, 5*) on the effects of topically applied fluoride on dental caries experience indicate that under the conditions of the application technique employed: (1) A series of four topical applications of a 2 percent solution of sodium fluoride, preceded by a single dental cleansing, effects a 40-percent reduction in dental caries incidence; more than four applications do not increase the caries-prophylactic effect. (2) The caries-inhibiting value of topically applied sodium fluoride is not appreciably decreased during a 3-year period following treatment. (3) The omission of dental cleansing prior to a series of applications reduces the effectiveness of topically applied sodium fluoride solution by approximately half. (4) Application of a saturated solution of lead fluoride (0.06 percent), using the same application technique as for solutions of sodium fluoride, is not associated with a significant reduction in the incidence of dental decay.

This report presents the results of seven additional studies designed to investigate the possibility of increasing the caries-inhibiting effect of topically applied fluoride solutions. Briefly, the results of these studies indicate: (1) The application of a 2 percent solution of sodium fluoride to the teeth, followed immediately by a 5 percent solution of calcium chloride, does not increase the caries-prophylactic effect over that accomplished by the use of a solution of sodium fluoride alone. (2) An increase in the time interval between applications of the fluoride solution in a given series of applications from one or two a week to one each 3 or 6 months decreases the caries-prophylactic effect observed. (3) A concentration of 1 percent solution of sodium fluoride appears to be equally as effective in inhibiting new dental decay as a 2 percent sodium fluoride solution.

MATERIALS AND METHODS

During the period from May to December 1946, groups of school children in Bradford, Tipp City, Newton, and Piqua, Ohio—all in Miami County—were given applications to the teeth in half the mouth, as follows:

Group 1. Two applications of a 2 percent solution of sodium fluoride followed by the application of a 5 percent solution of calcium chloride, using cotton applicator, and given at the rate of one or two treatments a week, without initial cleansing of the teeth.

Group 2. Two applications of a 2 percent solution of sodium fluoride followed by the application of a 5 percent solution of calcium chloride, using cotton applicator, and given at the rate of one or two treatments a week, with initial cleansing of the teeth.

Group 3. Four applications of a 2 percent solution of sodium fluoride followed by the application of a 5 percent solution of calcium chloride, using cotton applicator, and given at the rate of one or two treatments a week, with initial cleansing of the teeth.

Group 4. Three applications of a 2 percent solution of sodium fluoride, using cotton applicator, and given at the rate of one treatment each 3 months, cleansing of the teeth preceding each application.

Group 5. Two applications of a 2 percent solution of sodium fluoride, using cotton applicator, and given at the rate of one treatment each 6 months, cleansing of the teeth preceding each application.

Group 6. Two applications of a 1 percent solution of sodium fluoride, using spray bottle, and given at the rate of one or two treatments a week, with initial cleansing of the teeth.

Group 7. Four applications of a 1 percent solution of sodium fluoride, using spray bottle, and given at the rate of one or two treatments a week, with initial cleansing of the teeth.

In approximately half the children in each group, teeth in upper and lower right mouth quadrants were treated; in the other half of the children, teeth in left mouth quadrants were treated. Teeth in untreated mouth quadrants served as controls. Fine pumice paste and motor driven rubber cup were used for cleansing the teeth. A detailed dental examination was made and the findings recorded for each of the children before treatment was begun.

The examinations were made with a No. 4 plane mouth mirror and a double end No. 5 explorer, under artificial light and with compressed air available for use at the discretion of the examiner. The treatment consisted of isolating the teeth of the treated side with cotton rolls, drying with compressed air and wetting the crown surfaces with the treatment solution by using either cotton applicator or a fine spray. The applied solution was allowed to dry in air for from 3 to 4 minutes and then the cotton rolls were removed and the child dismissed.

In the case of the first three study groups, the crown surfaces of the teeth were wet with a 5 percent solution of calcium chloride after the solution of sodium fluoride had dried. The calcium chloride solution was also permitted to dry in air.

One year after the series of applications were begun, the children were reexamined. Analysis of the data on caries experience is confined to the erupted permanent teeth present at the time of the initial examination.

The age classification of the children in each of the seven study groups reported is shown in table 1. The number of males and females in each group is approximately equal.

FINDINGS

The caries experience during the study year in treated and untreated teeth of children included in each of the seven study groups is shown in tables 2 and 3.

Application of Sodium Fluoride and Calcium Chloride Solutions

The first three studies are concerned with the application of 2 percent solution of sodium fluoride and 5 percent solution of calcium chloride under various treatment combinations. These studies were conducted on the basis of the assumption that when the sodium fluoride solution is applied to the tooth enamel, the uncombined or excess fluoride ions might be used to protect the enamel further against the forces of dental caries by facilitating a surface deposition of calcium fluoride, particularly in the orfices of the enamel lamellae (*6*).

The children whose teeth had been given two applications of a 2 percent solution of sodium fluoride and a 5 percent solution of calcium chloride, without prior dental cleansing, were found to have 86 new carious teeth in treated quadrants and 78 in untreated quadrants at the end of the first study year. This negative result is within the range of variability that might be anticipated from only two applications not preceded by tooth cleansing.

When the teeth were cleansed prior to being wet with two applications of these solutions, there were, at the end of the study year, 216 new decayed teeth in treated quadrants as compared with 271 in untreated quadrants, or a reduction in new decay of 20.3 percent. After four applications of the two solutions, preceded by dental cleansing, 140 new carious teeth were observed in treated mouth quadrants as compared with 207 in untreated mouth quadrants —a reduction in new dental caries of 32.4 percent.

The caries-prophylactic effect of two applications, preceded by tooth cleansing, is equal to that observed after two applications of a 2 percent solution of sodium fluoride alone, using the same treatment technique. The reduction in new dental decay resulting from four applications of the combined solutions, preceded by tooth cleansing, is a little less than that resulting from four applications of a 2 percent solution of sodium fluoride alone (*5*).

Spacing of Applications

The treatment procedure used in applying topical fluorides has varied with investigators. In previous studies reported in this series, all applications were made within a period of several weeks at the beginning of the study at the rate of one or two treatments a week, preceded by a single dental cleansing. Other investigators (*7, 8*) have administered topical fluoride applications at the rate of one every 3 or 4 months, to a total of three or four applications in the series during a study year, and each application was preceded by dental prophylaxis.

Inasmuch as the number of topical fluoride applications, up to four, seems to be a basic consideration in the caries reduction observed, it might be assumed that the complete effectiveness of the treatment procedure would be postponed until four applications had been given. To determine the validity of this assumption, two studies were undertaken in which the series of applications were spaced at 3- and at 6-month intervals of time.

In the first of these (group 4) a total of three applications of a 2 percent solution of sodium fluoride was applied, one each 3 months, each application preceded by tooth cleansing. At the end of the study year 159 new carious teeth were found in treated mouth quadrants and 217 in untreated quadrants—a reduction in new dental decay of 26.7 percent.

In the second of the two studies conducted to determine the effect of wide spacing of applications (group 5), two applications of a 2 percent solution of sodium fluoride were applied, one at the beginning of the study and one 6 months later. The teeth were cleansed prior to each application. After one year, 193 sound teeth had become carious in treated, and 226 in untreated mouth quadrants—a reduction of 14.6 percent in new dental decay.

Concentration of Solution

In this series of studies, all solutions of sodium fluoride tested have been of 2 percent concentration. In study groups 6 and 7 the relative efficacy of a 1.0 percent solution has been explored. The treatment procedure was varied to include the use of a spray for application of the fluoride solution in place of the cotton applicator.

Two applications of a 1 percent solution were made within a week or two after dental cleansing, and after one year, 139 new carious teeth were observed in treated mouth quadrants, and 163 in untreated quadrants—a percentage reduction of 14.7 in new dental caries.

Table 1.—*Age distribution of Miami County, Ohio, school children examined at the end of a 1 year study period*

Study groups	All ages	Children by age at time of treatment										
		6	7	8	9	10	11	12	13	14	15	16
Group 1	208	......	17	30	25	36	30	24	18	22	6	......
Group 2	371	......	33	49	47	34	58	46	44	45	15	......
Group 3	272	......	33	45	33	42	27	33	28	26	5	......
Group 4	304	7	29	31	26	33	29	48	51	31	17	2
Group 5	362	8	47	45	47	49	48	56	48	12	1	1
Group 6	335	......	59	85	69	43	46	21	4	6	2	......
Group 7	276	16	30	46	33	28	46	32	29	13	3	......

TABLE 2.—*Dental caries experience during the 1-year period ending December 1947, in treated and untreated permanent teeth of Miami County, Ohio, school children*

Treatment groups by treated and untreated quadrants	Number of noncarious teeth (Dec. 1946)	New DF[1] teeth (Dec. 1947)	DF surfaces in new DF teeth	New DF surfaces in previously carious teeth	Total new DF surfaces
2% NaF—5% $CaCl_2$					
2 applications—no cleansing:					
Treated quadrant	1,489	86	90	59	149
Untreated quadrant	1,486	78	83	53	136
2 applications—prior cleansing:					
Treated quadrant	2,706	216	233	138	371
Untreated quadrant	2,736	271	301	127	428
4 applications—prior cleansing:					
Treated quadrant	1,927	140	155	86	241
Untreated quadrant	1,926	207	224	106	330
2% NaF, applications spaced, cleansing prior to each application:					
3 applications—one each 3 months:					
Treated quadrant	2,155	159	169	134	303
Untreated quadrant	2,192	217	240	159	399
2 applications—one each 6 months:					
Treated quadrant	2,376	193	211	167	378
Untreated quadrant	2,347	226	255	180	435
1% NaF, prior cleansing:					
2 applications:					
Treated quadrant	1,769	139	156	114	270
Untreated quadrant	1,772	163	182	125	307
4 applications:					
Treated quadrant	1,674	101	110	96	206
Untreated quadrant	1,680	166	185	118	303

[1] DF means carious (decayed or filled).

TABLE 3.—*Percentage reduction in new caries experience during the 1-year period ending December 1947, in the permanent, treated teeth of a group of Miami County, Ohio, school children*

Treatment groups	Percentage reduction in newly carious teeth
2% NaF—5% $CaCl_2$:	
2 applications—no cleansing	−10.3
2 applications—prior cleansing	20.3
4 applications—prior cleansing	32.4
2% NaF, applications spaced, cleansing prior to each application:	
3 applications—one each 3 mos	26.7
2 applications—one each 6 mos	14.6
1% NaF:	
2 applications—prior cleansing	14.7
4 applications—prior cleansing	39.2

Four applications of a 1 percent solution of sodium fluoride effected a reduction of 39.2 percent in new decay, with 101 new decayed teeth among those treated and 166 among teeth untreated.

SUMMARY

A study of the incidence of new dental decay in seven groups of Miami County, Ohio, children who received topical fluoride applications on the teeth in half the mouth has been presented and analyzed. The seven separate studies may be divided into three general groups:

1. Children whose teeth were given applications of 2 percent solution of sodium fluoride and 5 percent solution of calcium chloride, one group receiving two applications without prior tooth cleansing; another group, two applications with prior tooth cleansing, and the third group four applications with prior tooth cleansing. All applications were given at the rate of one or two a week at the beginning of the treatment period.

2. Children whose teeth were given two and three applications of 2 percent solution of sodium fluoride spaced intermittently over the first study year, each application preceded by a dental cleansing.

3. Children whose teeth were given by the spray method two and four applications of 1 percent solution of sodium fluoride, with prior tooth cleansing, all applications being given at the rate of one or two a week at the beginning of the treatment period.

Analysis of the data for the first study year indicates:

1. The use of calcium chloride as a supplemental treatment to applications with a 2 percent solution of sodium fluoride does not enhance the caries-inhibitive action of sodium fluoride alone.

2. An increase in the spacing between applications of a 2 percent solution of sodium fluoride from one or two weekly, to 3-month or to 6-month time intervals, decreases the observed caries-inhibiting action and apparently postpones the time when the full effectiveness of four applications is operative.

3. Apparently a 1 percent solution of sodium fluoride is as effective as a 2 percent solution. However, clinical experience with the caries-prophylactic effect of a 2 percent solution is at present far more extensive than with solutions of lower concentration.

4. Application of the fluoride solution to the teeth by means of a spray appears to be as effective as when application is made by cotton applicator.

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THE EFFECT OF TOPICALLY APPLIED FLUORIDES ON DENTAL CARIES EXPERIENCE. VII. Consolidated Report of Findings for Four Study Groups, Showing Reduction in New Decay by Individual Tooth and by Tooth Surface, and Frequency Distribution of Newly Decayed Teeth in Treated and Untreated Mouth Halves

By John W. Knutson and Grace C. Scholz

Previously reported studies in this series (*1–6*) have been concerned with the overall effect of topically applied fluorides on dental caries experience in the permanent teeth of children. In summary, these studies indicate:

1. A series of four topical applications of a 2 percent solution of sodium fluoride, preceded by dental cleansing, effects a 40 percent reduction in dental caries incidence. More than four applications do not increase the caries-prophylactic effect.
2. The caries-inhibiting value of topically applied sodium fluoride is not appreciably decreased during a 3-year period following treatment.
3. The omission of dental cleansing prior to a series of applications reduces the effectiveness of the fluoride applications by approximately one-half.
4. Application of a saturated solution of lead fluoride (0.06 percent), using the same application technique as for solutions of sodium fluoride, is not associated with a significant reduction in the incidence of dental caries.
5. The application of a 2 percent solution of sodium fluoride to the teeth, followed immediately by a 5 percent solution of calcium chloride, does not increase the caries-prophylactic effect accomplished by the use of a solution of sodium fluoride alone.
6. An increase in the time interval between applications of the fluoride solution in a given series of applications from one or two a week to one each 3 to 6 months decreases the caries-prophylactic effect observed.
7. A concentration of 1 percent solution of sodium fluoride appears to be equally as effective in inhibiting new dental caries as a 2 percent solution.

In four groups previously studied (*1, 5, 6*) a series of 4 or more applications of sodium fluoride solution effected approximately a 40 percent reduction in caries incidence during a one-year study period. Inasmuch as none of these studies is based on a large enough sample of children to determine reductions in dental caries by individual tooth type or tooth surface, the data for the four groups have been combined. The purpose of this study is to present for different kinds of teeth, and by tooth surface, the reduction in new caries effected by topical fluoride applications. In addition, the distribution of caries experience in fluoride-treated and untreated mouth halves is compared in order to determine the variation in individuals of the caries-prophylactic effect of topical sodium fluoride. Identification of the study groups according to previous report and the age classification of children in each group are presented in table 1.

In the first study group, teeth in the left side of the mouth are treated, while teeth in the right side of the mouth served as controls. In each of the three other study groups, approximately half of the children received treatment on teeth in the left side of the mouth and the other half on teeth in the right side of the mouth. Fine pumice paste and a motor driven rubber cup were used for cleansing the teeth. A detailed dental examination was made with plane mirror and explorer under artificial light and with compressed air available for use at the discretion of the examiner. The method of fluoride application consisted of isolating the teeth on the treated side with cotton rolls, drying with compressed air, and wetting the crown surfaces of the teeth with fluoride solution. The applied solution was allowed to dry in air for from 3 to 4 minutes; then the cotton rolls were removed and the child dismissed.

One year after the series of applications was begun, the children were reexamined. The examiner did not know which teeth had been treated or which were untreated controls. Analysis of the data on caries experience is confined to the erupted permanent teeth present at the time of the initial examination and fluoride application.

Table 1.—*Age distribution of four groups of school children examined 1 year after a series of fluoride applications had been made to the teeth in half the mouth of each child*

Number of applications	All ages	Children by age at time of treatment								
		7	8	9	10	11	12	13	14	15
Group No. 1[1]	288	10	18	26	41	39	50	44	50	10
Group No. 2[2]	225	17	18	32	31	31	28	25	26	17
Group No. 3[3]	259	35	30	26	40	40	31	25	27	5
Group No. 4[4]	260	30	46	33	28	46	32	29	13	3
Total	1,032	92	112	117	140	156	141	123	116	35

[1] 8–15 applications, 2 percent NaF, following initial cleansing, Arlington, North Mankato, St. Louis Park, Minn. (*1*).

[2] 4 applications, 2 percent NaF, following initial cleansing, Miami County, Ohio (*5*).

[3] 6 applications, 2 percent NaF, following initial cleansing, Miami County, Ohio (*5*).

[4] 4 applications, 1 percent NaF, following initial cleansing, Miami County, Ohio (*6*).

NEW CARIES REDUCTION IN ALL TEETH

The caries experience during a study year in fluoride-treated and untreated teeth of children in the four study groups is shown in table 2. At the time of initial examination approximately the same number of noncarious or sound permanent teeth were available in treated and untreated mouth quadrants. During the year, among the 1,032 children under study, the percentage reduction in caries attack on fluoride-treated as compared with untreated teeth was 40.3 percent. For the four studies separately, the reduction ranged from 38.7 percent to 41.7 percent.

NEW CARIES REDUCTION BY TOOTH

The measurement of percentage reduction in new caries, by comparing treated with untreated teeth, is based on the bilaterally equal occurrence of dental caries in left and right mouth quadrants of large groups of children. This bilateral

TABLE 2.—*Dental caries experience during a 1-year study period in fluoride-treated and untreated teeth of 1,032 children*

Quadrants	Initially noncarious teeth	Teeth becoming carious during study period	Percent attacked by caries
Treated:			
Upper	3,466	239	6.9
Lower	3,964	176	4.4
Total	7,430	415	5.6
Untreated:			
Upper	3,492	414	11.9
Lower	3,968	281	7.1
Total	7,460	695	9.3

symmetry in caries experience is also characteristic of homologous teeth on opposite sides of the mouth. Reduction in new decay, by tooth, is measured therefore by comparing the increment of caries in specific treated teeth with that in corresponding homologous teeth which were untreated.

Inasmuch as the increment of dental caries in certain teeth, such as lower incisors, is relatively small during a single year, even in the number of children included in this study, it is desirable to indicate which of the figures presented here can be considered statistically significant. For the purposes of this report, a probability of 0.0227 or less that an observed difference in rate of decay between teeth treated and untrated is due to chance is considered statistically significant.

The caries experience in two pairs of upper teeth (central incisor and cuspid) and in three pairs of lower teeth (central and lateral incisors, and cuspid) was relatively small, and the number of children in this study is not sufficiently large to establish that the observed caries reduction in these particular teeth was not due to chance (table 3). Less than 15 percent of total new decay occurring in upper teeth and only 7 percent of that occurring in lower teeth during a single study year affected these teeth.

Among the upper teeth for which the sample size is large enough to demonstrate statistical significance in the reduction in new caries, the range in reduction in treated teeth was from 34.7 percent in first molars to 50.9 percent in second bicuspids. In upper second molars, first bicuspids, and lateral incisors the reductions were 46.7, 46.8, and 50.7 percent, respectively.

In upper central incisors, a 22.7 percent reduction in new caries in treated teeth was found. The probability that this or a greater reduction is due to chance is 0.1515, and therefore is not statistically significant. Inasmuch as the mesial surface of the untreated central incisor is almost certain to be wet in most cases when fluoride solution is applied to the same surface of the homologous tooth in the treated half of the mouth, the amount of reduction in new decay observed in this tooth must be analyzed on the basis of this condition. When the analysis is made separately for the observed increment of new caries on mesial and on distal surfaces of the upper central incisor, it is found that mesial surface decay is slightly greater in treated than in untreated teeth, while distal surface decay was reduced approximately 40 percent.

Among lower teeth, the fluoride applications effected the lowest significant reduction in initial caries attack in first molars—22.2 percent, and the highest in first bicuspids—52.0 percent. New caries in lower second bicuspids was reduced 34.7 percent and in second molars 49.5 percent.

The number of teeth classified as sound or noncarious on initial examination and the proportion that became carious during a study year, by tooth type, and by treated and untreated mouth quadrants are shown in table 4.

TABLE 3.—*Percent less initial caries attack in fluoride-treated than in untreated teeth of 1,032 children, by specific tooth*

Teeth	Mouth quadrants		
	Upper	Lower	Both
Central incisor	22.7	44.4	26.4
Lateral incisor	*50.7	12.5	*46.8
Cuspid	33.3	66.7	41.7
First bicuspid	*46.8	*52.0	*48.6
Second bicuspid	*50.9	*34.7	*43.3
First molar	*34.7	*22.2	*28.7
Second molar	*46.7	*49.5	*48.1
All teeth	*42.3	*37.4	*40.3

*Statistically significant.

NEW CARIES REDUCTION BY TOOTH SURFACE

The reduction in new caries in all surfaces of fluoride-treated sound teeth in upper mouth quadrants for children in the four study groups was 40.6 percent, and in lower mouth quadrants the reduction was 34.1 percent (table 5).

In upper teeth, the reduction in new carious surfaces associated with topical fluoride applications was slightly greater for distal surfaces (45.1 percent) than for occlusal surfaces (42.4 percent). On mesial surfaces the initial caries attack was reduced 41.0 percent. This high proportionate reduction in new decay on interproximal surfaces is of particular interest since the dental cleansing which precedes topical fluoride applications is not a complete dental prophylaxis and the interproximal surfaces are not cleansed as thoroughly as the more accessible surfaces.

In upper mouth quadrants, more than half of the total new caries, by surfaces, in both treated and untreated teeth were occlusal surface caries. Approximately 40 percent of the total occurred on interproximal surfaces (mesial and distal). Buccal and labial surface caries was relatively negligible, and the 38.0 percent difference between treated and untreated for this surface classification is not statistically significant. A similar conclusion applies to the 14.3 percent reduction observed for the lingual surface.

In lower teeth, statistically significant reductions can be demonstrated only for new caries on occlusal surfaces—37.9 percent reduction, and on buccal and labial surfaces—50.0 percent reduction. More than three-quarters of total new decay in both treated and untreated teeth in lower mouth quadrants occurred on occlusal surfaces. Little or no reduction was noted for the mesial or distal surfaces of lower teeth. This latter finding is noteworthy since it may indicate a failure to wet these surfaces properly with the technique employed.

The detailed data on caries experience by tooth surface in treated and untreated mouth quadrants are shown in table 6.

FREQUENCY DISTRIBUTION OF NEWLY CARIOUS TEETH IN TREATED AND UNTREATED MOUTH HALVES

In the foregoing sections the caries reduction in individual teeth and on separate classes of tooth surfaces has been presented. The purpose of this section is to study the variation in effectiveness of the topical fluoride procedure on the teeth of individual children in the study group. In making this analysis the number of newly carious teeth occurring in each treated and untreated half of the mouth of each child is determined and the results arrayed in a frequency distribution.

The frequency distribution of the number of newly carious teeth within treated and untreated mouth halves for the 1,032 children in the four study groups is given in table 7. This distribution shows that the maximum number of carious teeth in the fluoride-treated side of the mouth is three and for the untreated half, five. Furthermore, the number of children with one, two, or three newly carious teeth in treated mouth halves is considerably less than those having equal numbers in untreated upper and lower mouth quadrants. On the other hand, the proportion of treated mouth halves in which no new decay occurred during the study year was substantially increased over the corresponding number of caries-free untreated mouth halves. In general, these findings indicate that the reduction in caries incidence effected by topical fluoride applications in a large group of children is the result of a fairly uniform lowering of the number of newly carious teeth each child would have had if the teeth had remained untreated.

This conclusion can be subjected to further examination by arbitrarily applying a 40-percent reduction to the caries experience observed in the control or untreated teeth of each child, arraying the results in a frequency distribution, and comparing this theoretical distribution with that observed for treated mouth halves. Under this method all children who actually experienced five newly carious teeth in the untreated group would be expected to have only three newly carious teeth had they been treated. Those actually experiencing four newly carious teeth would have two in most cases, but some would have three in order that the average be a 40-percent reduction. The resulting theoretical distribution is also presented in table 7. Comparison of the percent of mouth halves in each class according to number of newly carious teeth reveals a striking similarity between the treated, or observed, and the theoretical. Thus, this result supports the general conclusion that a series of four fluoride applications reduces dental caries incidence approximately 40 percent and that all children treated benefit to this extent.

TABLE 4.—*Number of initially noncarious teeth, and percent attacked by caries in fluoride-treated and untreated teeth of 1,032 children, by specific tooth*

Teeth	Mouth quadrants											
	Upper				Lower				Both			
	Initially noncarious teeth		Percent attacked by caries		Initially noncarious teeth		Percent attacked by caries		Initially noncarious teeth		Percent attacked by caries	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Central incisor	810	803	4.2	5.5	1,001	998	0.5	0.9	1,811	1,801	2.2	2.9
Lateral incisor	720	729	4.7	9.5	967	969	.7	.8	1,687	1,698	2.4	4.5
Cuspid	473	461	1.3	2.0	613	615	.2	.5	1,086	1,076	.8	1.1
First bicuspid	532	539	4.7	8.7	586	592	2.0	4.2	1,118	1,131	3.3	6.4
Second bicuspid	422	449	6.4	12.2	431	419	7.4	11.7	853	868	6.9	12.0
First molar	294	293	21.8	33.4	199	199	35.2	45.2	493	492	27.2	38.2
Second molar	215	218	22.7	42.2	167	176	29.2	55.1	382	394	25.7	48.0
All teeth	3,466	3,492	6.9	11.9	3,964	3,968	4.4	7.1	7,430	7,460	5.6	9.3

TABLE 5.—*Percent less initial caries attack in tooth surfaces of fluoride-treated than in untreated teeth of 1,032 children, by specific tooth surface*

Tooth surfaces	Mouth quadrants			Tooth surfaces	Mouth quadrants		
	Upper	Lower	Both		Upper	Lower	Both
Occlusal	*42.4	*37.9	*40.2	Buccal and labial	38.0	*50.0	*48.1
Mesial	*41.0	0	*31.3	All tooth surfaces	*40.6	*34.1	*37.9
Distal	*45.1	4.5	*35.5				
Lingual	14.3	100.0	22.6				

*Statistically significant.

TABLE 6.—*Number of initially noncarious tooth surfaces, and percent attacked by caries in fluoride-treated and untreated teeth of 1,032 children, by specific tooth surface*

Tooth surfaces	Mouth quadrants											
	Upper				Lower				Both			
	Surfaces initially noncarious		Percent attacked by caries		Surfaces initially noncarious		Percent attacked by caries		Surfaces initially noncarious		Percent attacked by caries	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Occlusal	3,466	3,492	3.9	6.8	3,964	3,968	3.4	5.5	7,430	7,460	3.7	6.1
Mesial	3,466	3,492	1.8	3,0	3,964	3,968	.8	.7	7,430	7,460	1.2	1.8
Distal	3,466	3,492	1.1	2.0	2,964	3,968	.5	.6	7,430	7,460	.8	1.2
Lingual	3,466	3,492	.7	.8	3,964	3,968	.0	.1	7,430	7,460	.3	.4
Buccal and labial	3,466	3,492	.1	.2	3,964	3,968	.6	1.1	7,430	7,460	.4	.7
All tooth surfaces	17,330	17,460	1.5	2.6	19,820	19,840	1.1	1.6	37,150	37,300	1.3	2.1

SUMMARY

Previously presented data relating to the reduced incidence of dental caries in fluoride-treated as compared with untreated permanent teeth of 1,032 children have been presented and analyzed separately for each tooth type and tooth surface. In summary, the analysis indicates that:

1. For the study group included in this presentation, the over-all reduction in newly carious teeth in fluoride-treated as compared with untreated teeth was 40.3 percent for teeth in upper mouth quadrants and 37.4 percent for teeth in lower mouth quadrants.

TABLE 7.—*Frequency distribution of number of initially carious teeth accrued during study year in treated and untreated mouth halves of 1,032 children*

Number of newly carious teeth	Number of mouth halves					
	Untreated		Treated		Theoretical*	
	Number	Percent	Number	Percent	Number	Percent
0	546	52.9	703	68.1	678	65.7
1	329	31.9	253	24.5	295	28.6
2	114	11.0	66	6.4	55	5.3
3	35	3.4	10	1.0	4	.4
4	6	.6	-----	-----	-----	-----
5	2	.2	-----	-----	-----	-----
Total	1,032	100.0	1,032	100.0	1,032	100.0

*Assuming newly carious teeth in untreated mouth halves reduced by 40 percent.

2. Among teeth in upper mouth qaudrants, except central incisors and cuspids, the reductions varied from 34.7 percent for first molars to 50.9 percent for second bicuspids.

3. Among teeth in lower mouth quadrants, except incisors and cuspids, the reductions varied from 22.2 percent for first molars to 52 percent for first bicuspids.

4. The over-all reduction in newly carious tooth surfaces in fluoride-treated as compared with untreated teeth averaged 37.9 percent—40.6 percent in upper mouth quadrants, and 34.1 percent in lower mouth quadrants.

5. For upper teeth alone, the reduction in new decay on distal surfaces exceeded that on occlusal surfaces (45.1 percent on distal; 42.4 percent on occlusal), while mesial surface decay was lowered as the result of fluoride applications by 41.0 percent

6. Comparison of the distribution of the number of newly carious teeth in treated and untreated mouth halves and a theoretical distribution calculated by applying a 40-percent reduction to the number of carious teeth observed in each untreated mouth half indicates that the caries prophylactic effect of topical sodium fluoride is remarkably uniform for individual children.

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STUDIES OF THE ACTION OF SODIUM FLUORIDE ON HUMAN ENAMEL BY ELECTRON MICROSCOPY AND ELECTRON DIFFRACTION

By DAVID B. SCOTT, ROBERT G. PICARD, and RALPH W. G. WYCKOFF

Clinical evidence accumulated over the past few years has indicated that the topical application of a sodium fluoride solution is effective in controlling dental caries (*1*). The mechanism by which the fluoride acts is as yet not known and is a matter of widespread laboratory investigation. Most studies to this end have been in the field of analytical chemistry, and they have shown that fluorine is taken up by powdered enamel suspended in solutions of sodium fluoride (*2*), and that after such treatment the powdered enamel is less soluble in certain dilute acids (*3, 4, 5*). Recent studies have demonstrated that electron microscopy, and X-ray and electron diffraction afford useful physical approaches to the problem. Gerould, by electron microscopy of silica replicas (*6*), has shown a deposit on enamel slabs after immersion for 30 days in 4 percent NaF. His X-ray and electron diffraction studies of fluoride-treated samples of powdered enamel have indicated that the product of the reaction is calcium fluoride. Holager and Syrrist (*7*), and Syrrist (*8*), have also reported that calcium fluoride is formed.

This report describes three sets of experiments designed to study by electron microscopy the visible effect of treating enamel with sodium fluoride, and to study by electron diffraction the crystalline changes on similarly treated enamel surfaces. The first series involves the electron microscopy of enamel slabs treated for varying lengths of time with NaF solutions; the second is devoted to the electron microscopy of enamel slabs etched with acid before and after treatment with NaF. In the third set of experiments, electron diffraction studies have been made of the crystalline composition of the surfaces of enamel slabs before and after similar treatments.

Previous studies (*9, 10, 11*) have shown that the outer surfaces of the enamel, which are those treated clinically, are rich in structural detail, and that this detail varies according to age of the tooth, the location of the surface on the tooth, and the position of the tooth in the dental arch. Because of these variations and the fact that our

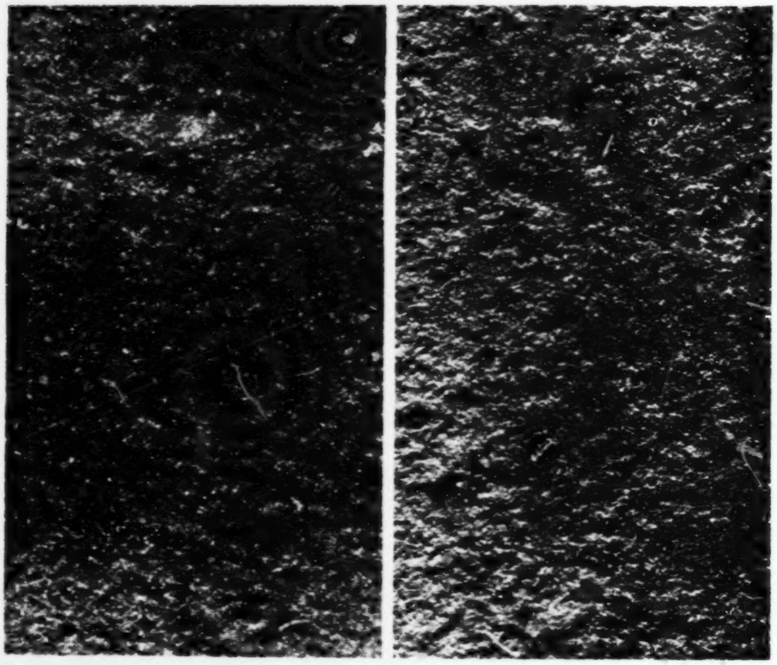

Figure 1.—*Left and right: minute roughness observed in most control specimens (7,200X).*

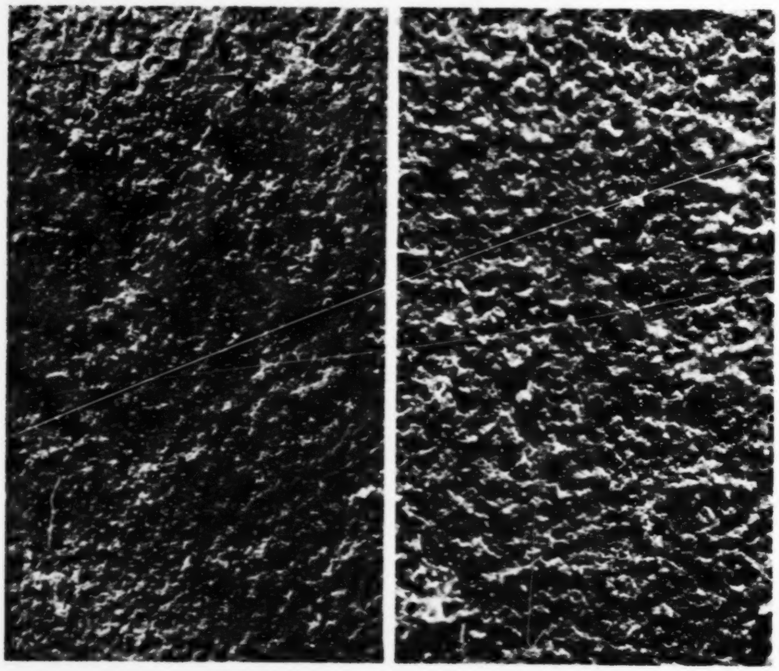

Figure 2.—*Left and right: more pronounced roughness observed in some control specimens (7,200X).*

knowledge of tooth surfaces is still largely restricted to detail visible at the low magnifications of the optical microscope, it has seemed best not to examine fluoride-treated outer surfaces in these first experiments. Instead all specimens have been slabs of subsurface enamel. The slabs, from noncarious molars, were so cut and polished (*12*) that their faces were approximately parallel to the outer enamel surfaces.

All test and control solutions were filtered and placed in paraffin-lined jars. Eight to twelve slabs were placed, polished face up, on the bottom of a jar containing 200 ml. of solution, and the closed jar was kept at room temperature for the required length of time. The slabs were then removed and rinsed for 1 minute by agitation in distilled water. At this point, in those experiments that required it, the slabs were etched and rinsed.

The slabs were next dried and collodion replicas made of the polished surfaces according to the usual techniques (*12*). These replicas were then shadowed with chromium at a 3:1 angle and examined and photographed with an RCA type EMU electron microscope. A total of about 1,200 preparations from the replicas were examined; observations were recorded in approximately 3,000 micrographs.

Electron diffration was conducted on intact slabs prepared and treated in the same manner as for electron microscopy. For examination these slabs were removed from the solutions, rinsed, dried, and placed immediately in the specimen holder of an RCA type EMD diffraction equipment.

ELECTRON MICROSCOPY OF ENAMEL TREATED WITH SODIUM FLUORIDE

In these experiments, enamel slabs from 261 teeth were immersed in sodium fluoride solutions for periods ranging from 5 minutes to 30 days. The number of specimens and length of treatment are shown below.

Immersion time	*Number of specimens immersed in* NaF	H_2O
5 minutes	30	12
16 minutes–2 hours	48	22
16–24 hours	39	21
2 days	20	10
7 days	12	8
15 days	18	12
30 days	94	68
Total	261	153

Control slabs, distributed as shown in the table, were immersed for the same periods of time in distilled water. All solutions were 2 percent NaF except in the experiment where slabs were immersed for 30 days. In this case, 20 specimens were immersed in 3 percent NaF and a like number in 4 percent NaF. The pH of the solutions was not adjusted, but measurements were made at the beginning and end of each experiment. The initial pH was always between 7 and 7.7; it did not change appreciably during the shorter experiments, but at the end of longer experiments the final pH was generally higher with a maximum of 8.5.

There has been considerable variation in the appearance of specimens subjected to the same treatment. These variations have existed not only between specimens but also between different areas on the same specimen. The controls have been relatively uniform, showing only minute roughness (fig. 1) except in a few instances where this roughness has been more pronounced (fig. 2). In the treated specimens, on the other hand, there have been great differences in appearance. About three-quarters of the specimens immersed in NaF for periods from 5 minutes to 24 hours closely resembled the controls (fig. 3). There were a few small areas on the remainder which, though slightly different in appearance from the controls, did not exhibit the pronounced changes noted in specimens exposed for 15 and 30 days. Half of the 2- and 7-day specimens appeared the same as the controls. About a quarter showed small patches like those seen after the shorted exposures, while the remaining quarter had small areas demonstrating the pronounced changes seen after the long immersions (fig. 4). A widespread, striking alteration in surface appearance was first noted after immerision for 15 days. About half of these specimens showed an inhomogeneous, apparently crystalline deposit (fig. 5). The crystals were distributed over the entire area observed, but were sufficiently separated to leave seemingly unaffected areas. The remaining 15-day specimens showed only small scattered areas of similar deposit. About two-thirds of the slabs immersed for 30 days showed crystalline deposits over the entire areas observed. The amount of deposit varied from that noted in the 15-day specimens to layers so heavy that no unaffected areas could be seen (fig. 6). Most of the remaining specimens showed only small areas of deposit; a few were negative and resembled the controls.

To determine whether stronger NaF solutions would produce a more plentiful deposit, 20 more specimens were immersed for 30 days in 3 percent NaF, and 20 in 4 percent NaF. The same variations in deposit were observed with these stronger solutions as with the 2 percent solution, and the effects did not appear more pronounced.

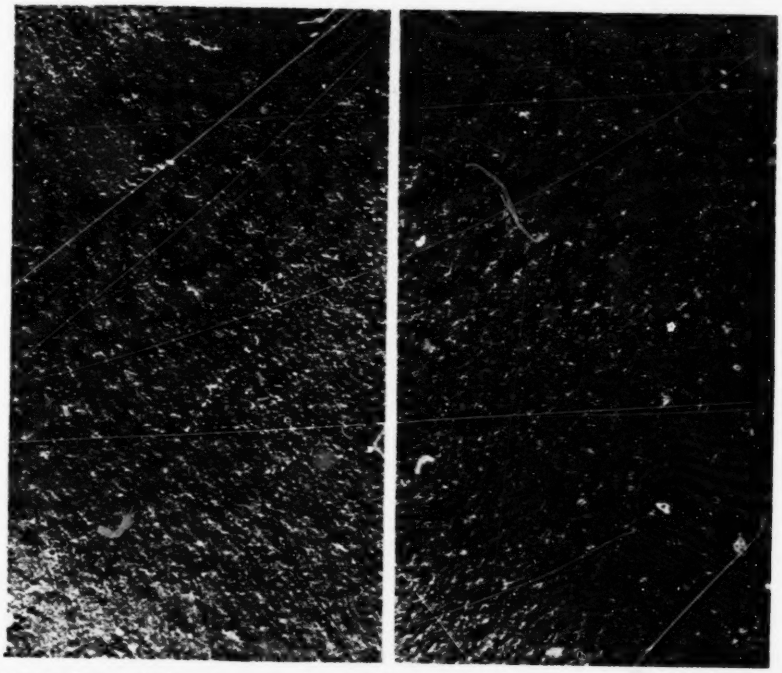

FIGURE 3.—*Typical appearance of enamel immersed in NaF for 5 minutes (left) and 24 hours (right) (7,200X).*

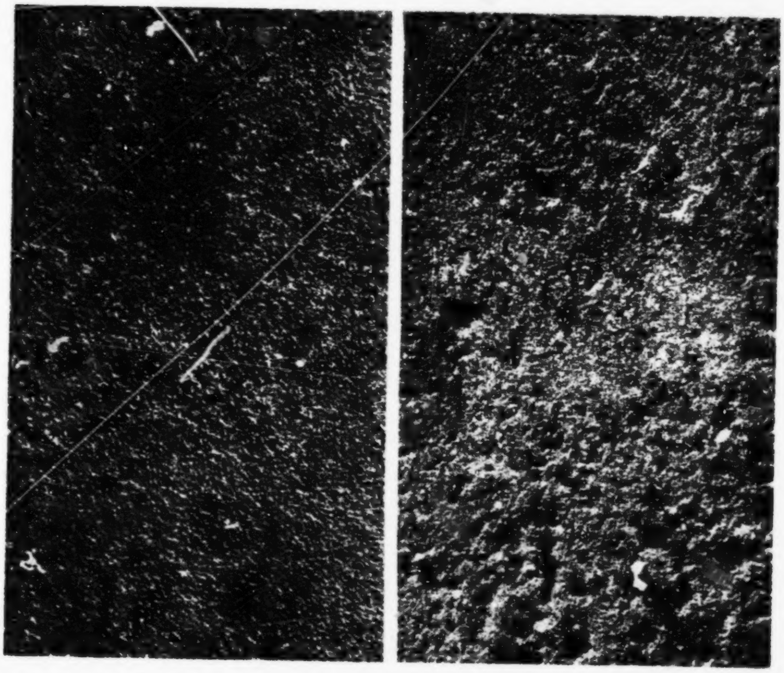

FIGURE 4.—*Normal (left) and slightly changed (right) areas on enamel after 2 and 7 days in NaF (7,200X).*

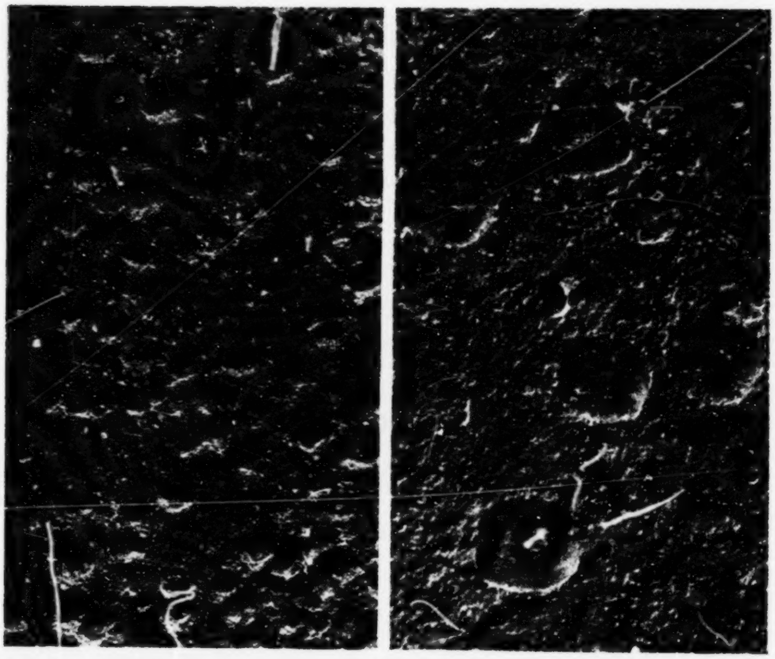

Figure 5.—*Deposit formed on enamel after 15 days in NaF. (Left, 7,200X; right, 11,000X).*

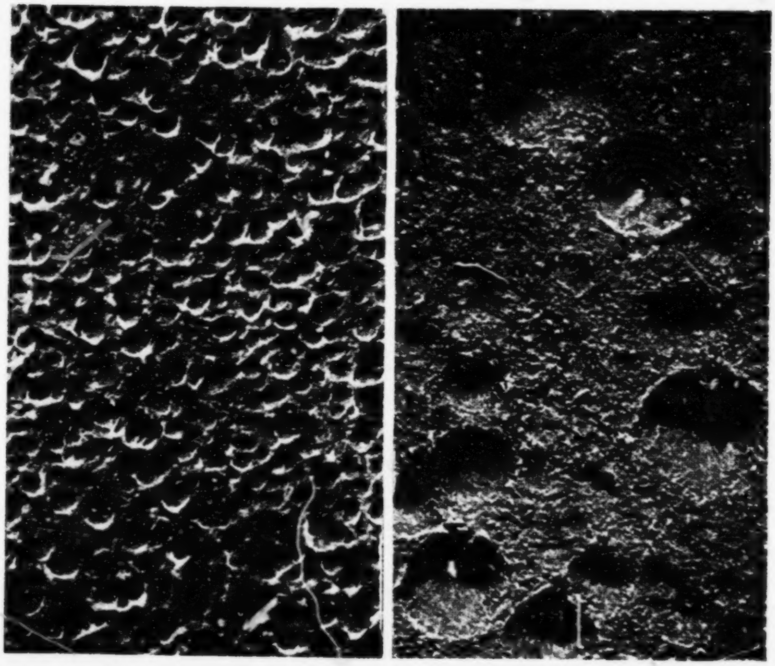

Figure 6.—*Deposit formed on enamel after 30 days in NaF. (Left, 7,200X; right, 11,000X).*

These experiments bring out the fact that prolonged treatments with NaF are required to alter the appearance of enamel as seen at the high magnifications of the electron microscope. They show that immersion for about 7 days in 2 percent NaF solutions is required to establish the first obvious traces of a surface deposit, and that the effect is progressively more pronounced at 15 and 30 days.

The permanence of the deposit was investigated through washing experiments. The test objects were 36 slabs which had been immersed for 30 days in 4 percent NaF. To serve as controls, a similar number of slabs were immersed in water and the same number in 5 percent NaCl for 30 days. After immersion, each group of slabs was placed in a separate glass vessel containing two liters of distilled water agitated by a mechanical stirrer. The water was changed daily, and 12 specimens were removed from each group after 1 day of washing, 12 after 3 days, and 12 after 6 days. A pronounced deposit remained on all the NaF-treated specimens which had been washed for 1 day (fig. 7, left); traces of deposit were observed after 3 days of washing (fig. 7, right), but no deposit was seen after 6 days of washing (fig. 8, left). Both the water and NaCl controls appeared normal after each period of washing (fig. 8, right). It thus appears from these preliminary experiments that the surface deposit which results from long immersion in NaF solutions is slowly removed by immersion in water. Subsequent studies are planned to investigate further the permanence of such deposits.

ELECTRON MICROSCOPY OF ENAMEL ETCHED BEFORE AND AFTER IMMERSION IN SODIUM FLUORIDE SOLUTIONS

It has previously been reported by Gerould (*6*) that a surface deposit is formed on enamel pre-etched with acid and immersed in NaF, and by Gerould (*6*), Holager and Syrrist (7), and Syrrist (*8*), that enamel becomes more resistant to acid after treatment with NaF. In the course of other studies in this laboratory on the fine structure of enamel, marked variations have been noted in its response to acid. The acid which has been most often used, and which has revealed much interesting fine detail, is 0.1 N HCl. Gerould (*6*), using silica replica techniques, and Syrrist (*8*), using a double replica method, etched enamel for 10 seconds in 0.1 N HCl, but this period seems to produce too great a depth of etch for satisfactory shadowed collodion replica studies. Although in many areas the resulting etch is sufficiently shallow to produce detail which can be visualized clearly, in most cases the elevations and depressions are so great, and their shadows so long, that much detail is obscured (fig. 9, left). We have found the most generally useful etch to be one obtained through 5 seconds contact with 0.1 N HCl. With this etch, it has been repeatedly observed that in different areas on a single specimen, and on different specimens, the enamel may be so etched as to produce a pronounced enamel rod pattern (fig. 9, right), a faint rod pattern (fig. 10, left), or even no apparent change in structure at all (fig. 10, right). This makes it necessary to exercise considerable caution in the interpretation of structural differences seen in etched fluoride-treated specimens.

A deposit similar to that noted on unetched specimens is formed when preetched specimens are placed in NaF solutions. This was demonstrated in an experiment in which 28 enamel slabs were etched for 5 seconds in 0.1 N HCl, rinsed by agitation in distilled water for one minute, and placed in 4 percent NaF. Twelve specimens were removed after 15 days and the rest after 30 days. A deposit was observed under the electron microscope on most of the surfaces, and it was present in amounts roughly similar to those seen on the unetched slabs. In many instances an enamel rod pattern could no longer be seen (fig. 11, left); in some areas it was only faintly visible (fig. 11, right), and in a few cases it was as pronounced as that found on the controls, with no trace of deposit.

In a second experiment, 20 slabs were etched for 5 seconds, and washed for 24 hours in a vessel containing 2 liters of distilled water agitated by a mechanical stirrer prior to immersion in the NaF. This was done in order to be certain that all of the soluble salts and debris were thoroughly removed from the etched surfaces. The specimens were then immersed for 30 days in 4 percent NaF, washed, and replicated as usual. The deposit was noted on most of these preparations, but their study left the general impression that it was less plentiful than before.

It has proved equally difficult to interpret the results of etching specimens subsequent to their immersion in fluoride solutions because of their varied appearance after etch. In one trial 30 specimens, immersed in 2 percent NaF for periods of 5 minutes to 40 hours, rinsed for 1 minute, and etched for 5 seconds with 0.1 N HCl, showed no deposit and had surface structures similar to those in etched untreated enamel. There was, however, some suggestion of an increased

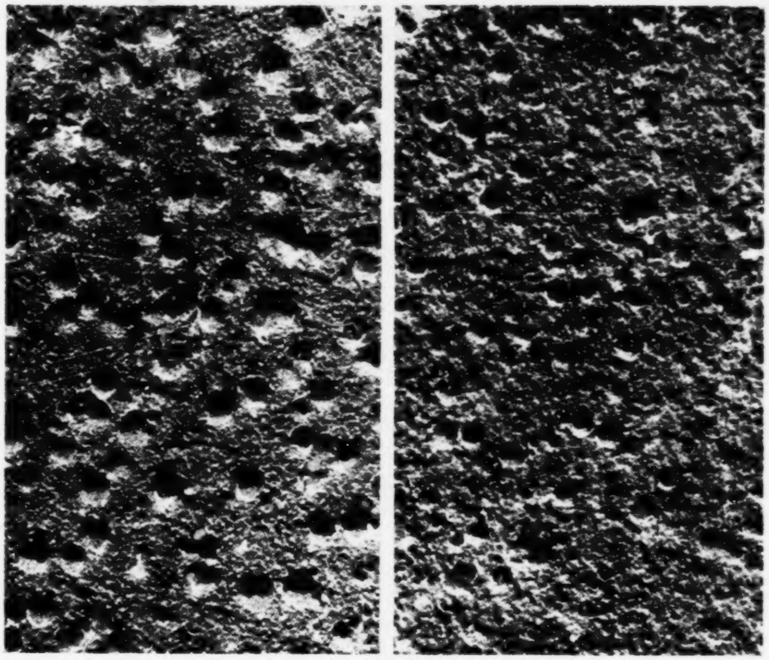

FIGURE 7.—*Appearance of 30-day NaF treated specimens after 1 day (left) and 3 days (right) washing (7,200X).*

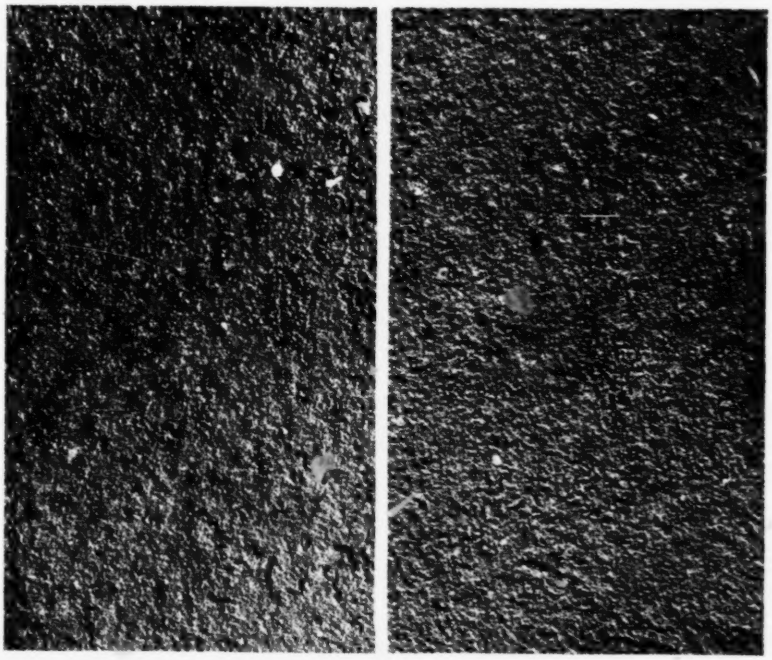

FIGURE 8.—*Appearance of 30-day NaF treated specimens (left) and controls (right) after 6 days washing (7,200X).*

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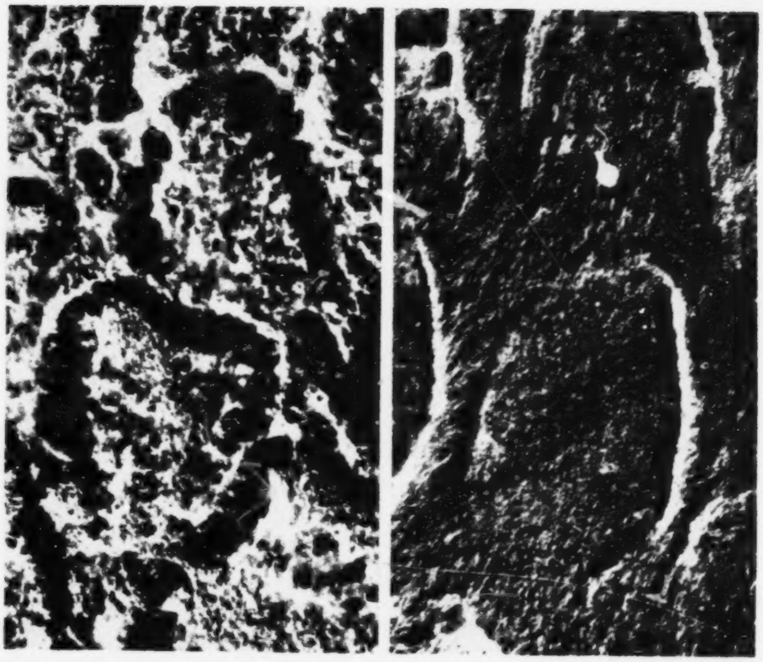

FIGURE 9.—*Left: untreated enamel etched 10 seconds with 0.1 N HCl. Right: untreated enamel etched 5 seconds with 0.1 N HCl (7,200X).*

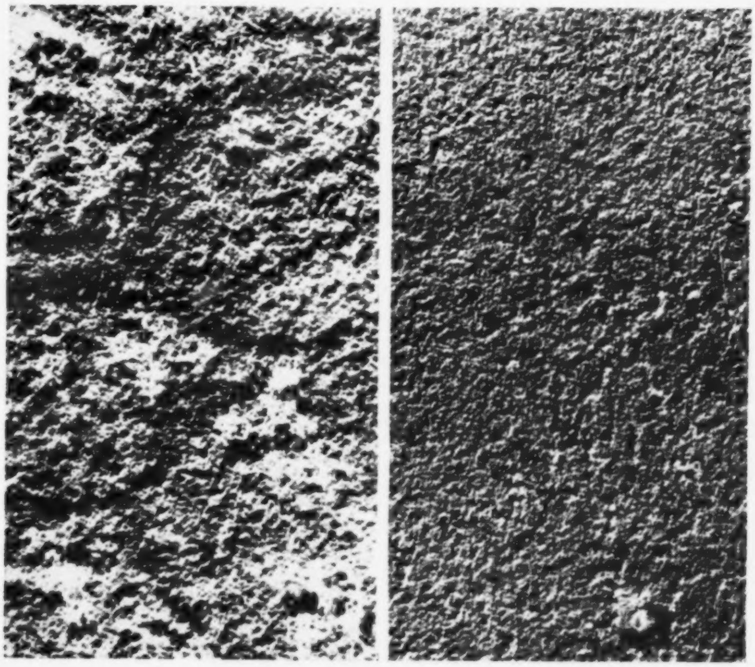

FIGURE 10.—*Untreated enamel etched 5 seconds with 0.1 N HCl. Left: faint rod pattern. Right: no change in structure. (7,200X).*

acid resistance of the surfaces of 20 other specimens which had been immersed for 30 days, and similarly etched. After etching, some specimens still showed traces of deposit (fig. 12, left), but other specimens appeared to be free of deposit (fig. 12, right), and a few showed areas of apparently normal etched enamel. It is evident that much more work must be done with etched normal and treated enamel before definite evidence can be obtained concerning changes in acid solubility brought about by NaF.

ELECTRON DIFFRACTION OF ENAMEL TREATED WITH SODIUM FLUORIDE

Although the electron microscope has revealed the formation of a visible deposit on enamel surfaces after prolonged immersion in NaF solutions, it has failed to show changes after the shorter treatments that are of clinical significance. Changes resulting from these short treatments can, however, be demonstrated through electron diffraction.

Taking advantage of the ease and precision with which the new RCA type EMD electron diffraction unit can provide electron diffraction patterns from the surfaces of solid objects, we have used it to follow chemical changes in the surfaces of enamel slabs which have been treated by NaF in a fashion identical to those studied by electron microscopy. Such diffraction studies of enamel surfaces have a two-fold advantage over diffraction studies using powdered enamel. They permit a direct comparison with the results of electron microscopy since patterns can be obtained from a surface before and after treatment, and from the exact surface that is to be replicated for microscopy. They also allow determination of chemical changes that may be brought about in enamel by the relatively short exposures to NaF that are clinically feasible.

In a first diffraction experiment, 30 enamel slabs were immersed in 2 percent NaF and 10 in distilled water, both for 30 days. At the end of this time, the specimens were washed for 1 minute in water, dried, and electron diffraction patterns made from their surfaces. The pattern obtained from untreated enamel was that of apatite (fig. 13, left), while a completely different pattern was obtained from the specimens treated with NaF (fig. 13, center). This pattern has been identified as that of CaF_2 both by measurement of the observed spacings and by comparison with patterns from CaF_2 itself (fig. 13, right). The CaF_2 pattern was given by all the treated specimens, although, when replicas of the surfaces of the slabs were examined under the electron microscope, the usual variety in appearance and quantity of visible deposit was noted.

After experimenting with progressively shorter immersion periods in NaF, it was found that the apatite pattern is replaced by that of CaF_2 after as short an exposure as 3 minutes. This was demonstrated through an experiment in which patterns were taken from the surfaces of 28 enamel slabs before and after 3 minutes' immersion in 2 percent NaF; in all cases the CaF_2 pattern was present after the treatment. Replicas of 12 of these surfaces were then examined under the electron microscope and no deposit could be detected.

Preliminary washing experiments were performed similar to those in the electron microscopic studies. Patterns were taken from the surfaces of 12 specimens before and after a 3-minute immersion in 2 percent NaF. They indicated that the surface of every specimen had become microcrystalline CaF_2. These slabs were then washed in circulating water and new patterns made after 15 and 90 minutes. The CaF_2 pattern was invariably present after the shorter period, but after 90 minutes every pattern had reverted to the apatite pattern obtained before treatment with NaF.

It is apparently more difficult to bring about the reversion of the CaF_2 pattern to the apatite pattern in specimens which have been immersed for long periods in NaF. Thus 12 specimens, which gave CaF_2 patterns after 30 days' immersion in 2 percent NaF, were washed for 6 days in water and, at the end of this time, 9 of the slabs still yielded the CaF_2 pattern while the other 3 gave that of apatite.

These observations demonstrate that there is a definite modification of the surface of enamel treated with NaF for periods as short as 3 minutes. Taken in connection with the microscopic studies, they show that this alteration is superficial and that prolonged exposures are necessary before definite visible changes in surface structure can be recognized, even under the high magnifications of the electron microscope. These facts, together with the relatively short time required to wash it away, clearly show the thinness of the CaF_2 layer that is formed after short treatments with NaF.

SUMMARY

Electron micrographs have been made of replicas of the surfaces of enamel slabs treated with NaF solutions for lengths of time between 5 minutes and 30 days. Few visible surface changes were recog-

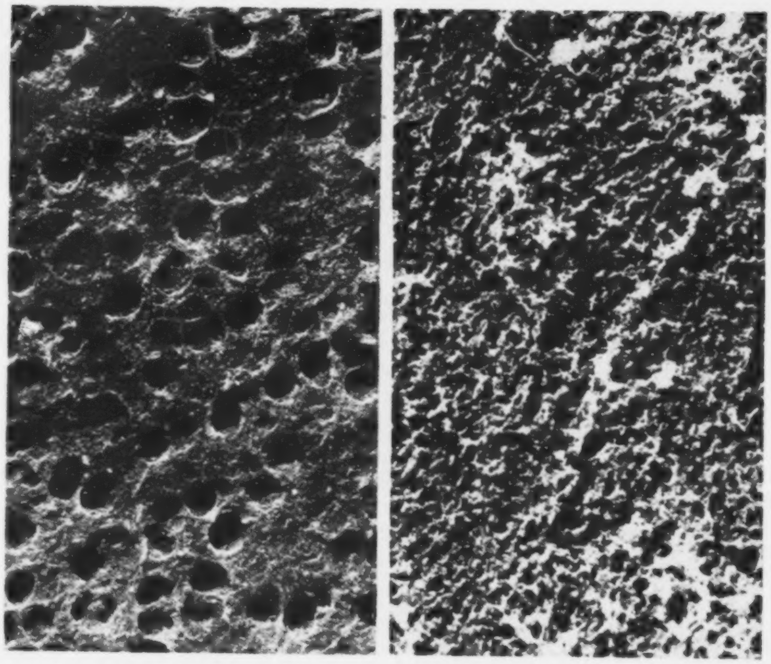

FIGURE 11.—*Pre-etched enamel immersed in NaF for 30 days.* (7,200X).

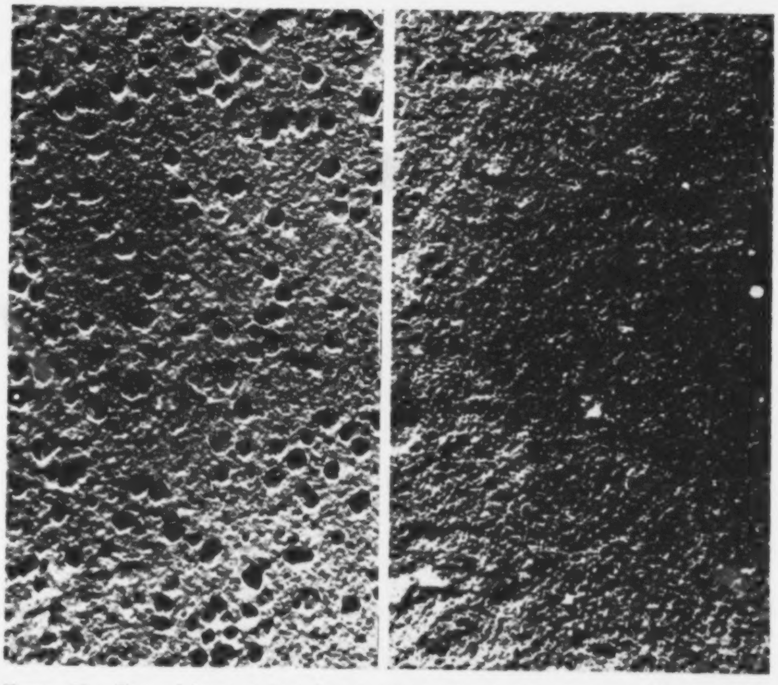

FIGURE 12.—*Enamel immersed 30 days in NaF and etched 5 seconds with 0.1 N HCl.* (7,200X).

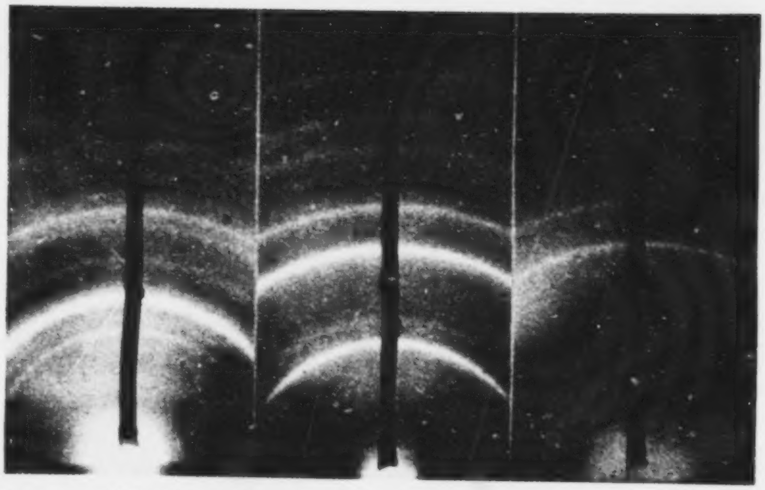

FIGURE 13.—*Diffraction patterns from untreated enamel (left); from enamel treated with NaF (center); and from CaF_2 (right).*

nized when the treatment was less than a week. Small amounts of deposit were noted after 7 days. Greater quantities were seen after 15- and 30-day treatments. It was not visible after the prolonged washing of 30-day treated specimens. Preliminary but inconclusive tests were made of the relative acid solubilities of untreated enamel and enamel after immersion in NaF.

Electron diffraction patterns have been made from the surfaces of enamel slabs before and after immersion in NaF for periods from 3 minutes to 30 days. In all instances the original apatite pattern was converted to that of CaF_2. The CaF_2 pattern reverted to that of apatite when specimens treated for 3 minutes with NaF were washed for 90 minutes in water, but when specimens treated for 30 days were washed for 6 days the reversion did not occur in all instances. Electron micrographs of surfaces after diffraction showed amounts of deposit to be expected from the treatments they had undergone.

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THE EFFECT OF TOPICALLY APPLIED FLUORIDES IN DENTAL CARIES EXPERIENCE ON CHILDREN RESIDING IN FLUORIDE AREAS

By R. A. DOWNS and W. J. PELTON

Previously reported studies by Knutson and others have been concerned with the over-all effect of topically applied fluorides on dental caries experienced in the permanent teeth of children who resided in nonfluoride areas (*1, 2, 3, 4, 5, 6, 7, 8*). Up to the present time, the data indicate that a series of four applications of 2 percent sodium fluoride over a short period of time will reduce the incidence of new caries by 40 percent in children who have a relatively high decay rate. The question arose in the Western States whether topical applications of sodium fluoride are as effective in children who reside in fluoride areas and have a relatively low decay rate as they are in nonfluoride areas where decay rates are high. Furthermore, it was desirable to know whether children reared in optimal fluoride areas, having one part per million fluorine in the water supply, would receive less benefit from topical applications of sodium fluoride as the children who resided in a community having less, but some, fluorine in its water supply. It seems unwise in the interest of sound public health practice to encourage either the public or the dental profession to use this method of caries control routinely in fluoride areas without having more specific research and information on the matter.

Accordingly, Sterling, Colo., having four-tenths parts per million fluorine in its public water supply, and Cheyenne, Wyo., having one part per million fluorine in its public water supply, were selected as test areas. Furthermore, previous dental examinations indicated that the children in these communities had a relatively low dental caries attack rate.

METHOD OF STUDY

In Sterling, the water histories of the school children were examined and about 300 who showed evidence of having resided continuously in the community all their lives were selected. Dental examinations of the children were performed in suitable school rooms using a portable and adjustable dental chair, natural light supplemented by a portable light, when necessary, and using new mouth mirrors and sharp explorers. The findings for each individual tooth and surface were recorded on a form according to accepted technics (*8*). After the examination the children were recalled and given four applications of sodium fluoride in the manner advocated by Knutson (*9*). The upper and lower teeth on the left quadrants of the mouth were treated and the opposite quadrants were used as controls. The examinations were made in October of 1948 and the series of four topical applications were completed in the next few weeks.

In Cheyenne during January, 1949, approximately 300 ninth grade children were selected according to their water histories and as in Sterling were examined and the data recorded. The left quadrants of the mouths of the Cheyenne children were treated in January, 1949, by the topical fluoride team assigned to the Wyoming State Health Department. The right quadrants were used as controls as previously.

The age range of the children in Sterling was from six years to eighteen years. In Cheyenne the age range was from twelve to sixteen years at the beginning of the study. In January, 1950, re-examinations of the Sterling children were made under the same physical conditions and the data recorded in the same manner as in the initial examinations. In February, 1950, re-examinations of the Cheyenne children were done under similar circumstances.

FINDINGS

All of the differences observed are well within the limits of chance variation and might have been seen had the children been given no treatment at all.

Table I shows the data for the treated and untreated quadrants of the permanent dentition that were present in the mouths of 285 Sterling, Colorado, and 253 Cheyenne, Wyoming, children. The data show that of 1,754 caries free teeth in the mouths of the Sterling children in the treated quadrants, there were 112 new carious teeth at the close of the study period. This represents 6.4 percent of the total number of non-carious teeth in the treated quadrants that became carious during the study period. The corresponding data of the untreated quadrants in the Sterling, Colo., children's mouths is 105 divided by 1,748, or 6.0 percent. It is seen from these two percentage figures that the treated quadrants had slightly more teeth become carious during the study period than the untreated quadrants. The total number of carious surfaces which became involved during the study period (161 compared to 146) also reflects this finding.

	No. of noncarious teeth as of Oct. 1948-Sterling, Jan. 1949-Cheyenne	No. of new carious teeth Jan. 1950-Sterling, Feb. 1950-Cheyenne	Carious surfaces in newly carious teeth	New carious surfaces in previously carious teeth	Total new carious surfaces
Sterling, Colorado, 0.4 ppm F					
Treated (Left)	1,754	112	131	30	161
Untreated (Right)	1,748	105	120	26	146
Cheyenne, Wyoming, 1.0 ppm F					
Treated (Left)	2,966	88	102	28	130
Untreated (Right)	2,948	103	112	34	146
Total of Study Cities					
Treated (Left)	4,719	200	233	58	291
Untreated (Right)	4,696	208	232	60	292

Dental caries experience during a one-year period ending in February, 1950, for the permanent teeth in the fluoride-treated and untreated quadrants of mouths of 285 Sterling, Colorado, and 253 Cheyenne, Wyoming, children.

Of 2,966 caries free teeth in the treated quadrants of the permanent teeth of the children in Cheyenne, Wyo., at the beginning of the study, 83 became carious during the experimental period. The 83 teeth represents three percent of the total number of caries free teeth which succumbed to the risk of becoming carious during the study period. The untreated quadrants showed that 103 teeth became carious out of 2,948 caries-free teeth at the beginning of the study, or 3.5 percent of the total number of teeth exposed to the risk of becoming carious.

The total number of new carious surfaces in the teeth of the Cheyenne children that became carious during the study period and the new surfaces that became carious in previously carious teeth, are 130 and 146. The data concerning new carious teeth and new carious surfaces in the treated and untreated quadrants support each other.

DISCUSSION

It should be obvious from this data that no reduction in the incidence of new caries in the permanent teeth of children residing in fluoride areas can be predicted following the application of sodium fluoride. This information should be regarded as inconclusive until further work is done with larger samples.

As a sidelight to these studies, it is interesting to note that in Sterling, Colo., the teeth decayed at the rate of .76 teeth per child per study period as compared to .75 teeth per year in Cheyenne. Although the study period was two months longer in Sterling than in Cheyenne, this and other data (*10*) suggest that the dental caries prevalence in communities having approximately one-half part per million fluorine per million parts of water is practically as low as in the communities having the heretofore regarded optimal amount of one part per million fluorine. The thought here is that the critical point at which fluorine is effective in reducing dental caries may be a great deal less than reported by Dean and his study of thirteen cities in four states (*11*).

SUMMARY

This study casts doubt as to the efficacy of topical applications of two percent sodium fluoride applied to the teeth of children born and reared in fluoride areas.

In the light of this study it would appear unsound, from either a monetary or personnel standpoint, to foster the routine use of topical fluoride treatments as a public health measure in cities having a concentration of fluorine in their public water of 0.4 p.p.m. or more. At the present time it appears reasonable to encourage the use of topical applications of sodium fluoride on the teeth of those children residing in a fluoride area but who have calcified their teeth on fluoride deficient waters.

It is believed, however, that before final decisions are made regarding the matter that further studies in fluoride areas, using a larger number of children, be undertaken to determine whether or not these findings are confirmed.

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TOPICAL APPLICATIONS OF FLUORIDE SOLUTIONS IN DENTAL CARIES CONTROL

F. E. Law, M. H. Jeffreys and H. C. Sheary

Both sodium fluoride and stannous fluoride in solutions of various concentrations have been used as topical agents, and their effectiveness as dental caries preventives among school children has been reported. A series of four applications of 2 percent solution of sodium fluoride to the teeth of children has been shown to reduce the incidence of dental caries by approximately 40 percent (*1*, *2*). Reports on the effectiveness of 2 and 8 percent concentrations of stannous fluoride solution, topically applied, have varied widely among different workers (*3–7*).

This study was designed to explore the relative caries-inhibiting effectiveness of sodium fluoride and stannous fluoride solutions under like controlled conditions. To establish comparability between this investigation and those conducted earlier, corresponding concentrations and procedures were chosen. The three study groups compared results observed after four applications of 2 percent sodium fluoride solution, four applications of 2 percent stannous fluoride solution, and one application of 8 percent stannous fluoride solution.

Participants were elementary school children residing in five Delaware communities. The water supplies in these areas were essentially fluoride free. A residence history was obtained for each individual, and children with more than 2 years' absence from the community at any time were omitted from the final evaluation. The residence requirements were established to insure that participants had not received the benefits of fluoride-bearing water.

MATERIALS AND METHODS

There were 884 children 7 to 13 years of age in Sussex and Kent Counties, Del., who met the residence requirements for participation in the study. Of this number, 823 children received the prescribed treatments and were present for the final 12-month reexamination. Study groups were set up by school so as to produce approximately equal numbers of children for participation in the evaluation of each of the three fluoride application procedures to be used (table 1). The three study groups showed similar rates of prevalence of dental caries at the beginning of the study.

A dental prophylaxis preceded the initial examination. All examinations were made by the same examiner using a No. 4 plane mouth mirror, double-end No. 5 explorer, artificial light, and portable dental chair, with compressed air available as required. Dental prophylaxis was not given prior to the second examination.

The 2 percent sodium fluoride solution was prepared daily. The stannous fluoride solutions (2 and 8 percent) were prepared four times daily because this compound is unstable in aqueous solution and susceptible to chemical change. Distilled water was used in the preparation of all solutions.

The first group of children received four applications of 2 percent sodium fluoride solution. The second group received four applications of 2 percent stannous flouride solution. Applications were made at 2- to 7-day intervals. The third group received one application of 8 percent stannous flouride solution. In each group one side of the mouth was treated and the other half provided the control. Approximately one-half of the children in each group were treated in right mouth quadrants and the remainder in left mouth quadrants. The application technique followed the method described by Knutson (*8*).

TABLE 1.—*Age distribution of 823 children available for reexamination at the end of 12-month period, by procedure*

Study procedure	All ages	Age, in years, at time of treatment						
		7	8	9	10	11	12	13
4 applications 2 percent sodium fluoride	269	31	50	61	52	38	22	15
4 applications 2 percent stannous fluoride	273	28	43	65	50	45	30	12
1 application 8 percent stannous fluoride	281	42	54	52	37	41	32	23

TABLE 2.—*Dental caries experience of 823 children during a 12-month period in permanent teeth of fluoride-treated and untreated mouth quadrants*

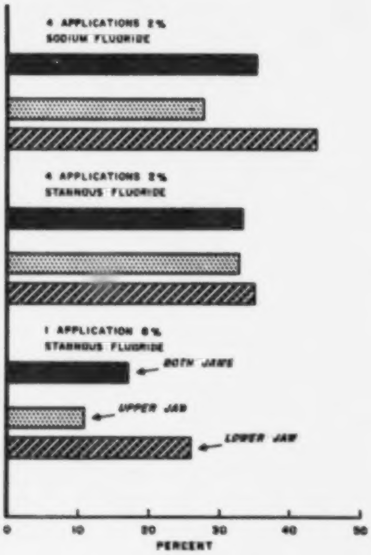
Study procedure	Number sound teeth May 1957	New DF[1] teeth May 1958		New DF[1] surfaces May 1958	
		Number	Percent	Number	Percent
	Both jaws				
4 applications 2 percent sodium fluoride:					
Treated quadrants	1, 526	103	6. 7	118	7. 7
Untreated quadrants	1, 540	159	10. 3	184	11. 9
4 applications 2 percent stannous fluoride:					
Treated quadrants	1, 542	100	6. 5	117	7. 6
Untreated quadrants	1, 562	151	9. 7	171	10. 9
1 application 8 percent stannous fluoride:					
Treated quadrants	1, 601	134	8. 4	148	9. 2
Untreated quadrants	1, 630	165	10. 1	197	12. 1
	Upper jaw				
4 applications 2 percent sodium fluoride:					
Treated quadrants	672	62	9. 2	70	10. 4
Untreated quadrants	669	85	12. 7	100	14. 9
4 applications 2 percent stannous fluoride:					
Treated quadrants	688	64	9. 3	73	10. 6
Untreated quadrants	686	95	13. 8	104	15. 2
1 application 8 percent stannous fluoride:					
Treated quadrants	716	80	11. 2	85	11. 9
Untreated quadrants	738	92	12. 5	109	14. 8
	Lower jaw				
4 applications 2 percent sodium fluoride:					
Treated quadrants	854	41	4. 8	48	5. 6
Untreated quadrants	871	74	8. 5	84	9. 6
4 applications 2 percent stannous fluoride:					
Treated quadrants	854	36	4. 2	44	5. 2
Untreated quadrants	876	56	6. 4	67	7. 6
1 application 8 percent stannous fluoride:					
Treated quadrants	885	54	6. 1	63	7. 1
Untreated quadrants	892	73	8. 2	88	9. 9

[1] Decayed or filled.

TABLE 3.—*Percent less new caries in fluoride-treated than in untreated previously sound permanent teeth of 823 children during a 12-month period*

Study procedure	Upper jaw	Lower jaw	Both jaws
	Percent less newly carious teeth		
4 applications 2 percent sodium fluoride	27. 6	43. 5	35. 0
4 applications 2 percent stannous fluoride	32. 6	34. 4	33. 0
1 application 8 percent stannous fluoride	10. 4	25. 6	16. 8
	Percent less newly carious surfaces		
4 applications 2 percent sodium fluoride	30. 2	41. 7	35. 3
4 applications 2 percent stannous fluoride	30. 3	31. 6	30. 3
1 application 8 percent stannous fluoride	19. 6	28. 3	24. 0

Percent less newly carious teeth in fluoride-treated than in untreated permanent teeth, by procedure



RESULTS

The dental caries experience of the 823 children during a 12-month period is shown in table 2.

In untreated mouth halves the incidence of new dental caries was remarkably similar in the three study groups during the 1-year study period. The percentage of untreated sound permanent teeth becoming carious ranged, by group, from a low of 9.7 percent to a high of 10.3 percent.

In treated mouth halves the percentage of new dental decay in the course of the study year was approximately equal in two of the study groups: for children treated with 2 percent solution of sodium fluoride, 6.7 percent; and for those with 2 percent solution of stannous flouride, 6.5 percent. The incidence of new caries was 8.4 percent for the group treated with 8 percent stannous fluoride solution.

The experience in the three study groups was similar for upper and lower teeth separately, although the rate of new dental caries incidence was somewhat higher in upper than in lower teeth in both treated and untreated mouth quadrants. In each treatment group the number of newly decayed tooth surfaces in previously sound permanent teeth followed a pattern similar to that of the newly carious teeth (table 2).

Percentage reductions in the incidence of newly carious teeth and of newly carious surfaces in previously sound teeth are shown in table 3 and the chart.

There were 35 percent fewer new carious teeth in treated as compared with untreated mouth quadrants following four applications of 2 percent sodium fluoride solution and 33 percent fewer after four applications of 2 percent stannous fluoride solution. The reduction was substantially less, 16.8 percent, for teeth treated with a single application of 8 percent stannous fluoride solution.

The topical fluoride study in Delaware shows that all fluoride-treated mouth quadrants incurred a lower incidence of new caries than untreated quadrants. The most favorable results were obtained from the two procedures using four applications of either 2 percent sodium fluoride or 2 percent stannous fluoride, as demonstrated by the greater relative reductions in new dental caries in treated teeth. At the 5 percent level of significance the reductions observed are greater than would be considered likely to have arisen by chance. Although permanent teeth treated with a single application of 8 percent stannous fluoride showed a lower caries rate than untreated teeth, the reduction was substantially smaller than in the other treatment groups, and it could not be concluded that the observed reduction was not due to chance.

SUMMARY

The effectiveness of sodium fluoride and stannous fluoride as dental caries preventives was tested in three groups of school children 7–13 years of age. One group received four applications of 2 percent sodium fluoride solution, another group was treated with four applications of 2 percent stannous fluoride solution, and a third group received one application of 8 percent stannous fluoride solution. In each group one side of the mouth was treated and the other served as control.

Results of this study, 12 months after the initial examination, indicate that the incidence of dental caries was significantly lower in non-carious permanent teeth treated with four applications of 2 percent sodium fluoride solution or 2 percent stannous fluoride solution than in untreated teeth. Their effectiveness was essentially the same.

One application of 8 percent stannous fluoride solution was considerably less effective in preventing dental caries than either of the other two procedures. The reduction in the incidence of dental caries obtained by this procedure was not statistically significant.

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PHYSIOLOGICAL EFFECTS OF FLUORIDE

The Health Status of Human Populations Exposed to Fluoride Drinking Water

A census of the natural fluoride content of community water supplies in the United States, was prepared for the Public Health Service by the Dental Directors of all State health agencies (Public Health Service Publication No. 655, Washington, D.C., 1959). Enumerated were 1,903 communities having at least one water source with 0.7 p.p.m. or more of natural fluoride. Upwards of 7,000,000 persons were living in these communities in 1947. "Natural fluoride water concentrations averaging 0.7–1.1 p.p.m. were present in 895 communities with about 4,256,000 residents." These quantities are optimum for dental caries control in the United States. While the duration of the exposure to natural fluoride waters by these millions of people has varied extensively, Colorado Springs, Colorado for example, has had a uniform and verified history of drinking water containing 2.5 p.p.m. fluoride within narrow limits, for some 75 years.

The health status of these millions of individuals using community fluoride drinking waters has been resolved to a major extent by epidemiological and clinical studies applied to representative population groups. It has been essential to evaluate particularly, the possibilities of commulative toxic skeletal fluorosis. In these regards the quantities of fluoride ingested in drinking water containing 1.00 p.p.m. of fluoride, are known to be eliminated efficiently from the body by urinary excretion. Skeletal tissues likewise have attainable tolerance levels of accumulated fluoride. No health hazard has been associated with the optimum low concentration of water-borne fluoride, which is known to reduce dental caries so effectively.

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KAJ ROHOLM, 1902–1948

Dr. Kaj Roholm was the author of the widely read monograph "Fluoride Intoxication, a Clinical-Hygienic Study with a Review of the Literature and Some Experimental Investigations," published in 1937. This renowned monograph is a classic of the fluoride literature. First to study in detail the effects on the human body of excessive quantities of fluoride, Dr. Roholm described numerous case histories and presented autopsy data on individuals exposed to dust-borne cryolite, during the mining and processing of this fluoride mineral. In addition, the monograph includes results of numerous experimental observations made by Dr. Roholm on animals ingesting fluorides. The early fluoride literature, particularly those articles published in Europe, was carefully documented and reviewed.

Dr. Roholm was born in Copenhagen, Denmark, on October 7, 1902. He was a medical graduate, with honors, from Copenhagen University in 1928, was granted a Master of Public Health degree in 1933, and received his Doctor of Medicine from the same University in 1935. His thesis was titled "Fluorine Intoxication." An acknowledged specialist in both internal medicine and metabolic diseases, Dr. Roholm became Deputy City Health Officer of Copenhagen in 1940 and held this position until the time of his death on March 28, 1948.

I became acquainted with Dr. Roholm during an all too limited period, when he visited this country in 1945. He was a particularly dedicated, mild-spoken man, modestly recalling his elaborate clinical studies and his review of the fluoride literature. He continued this work under the extremely difficult circumstances of World War II. After corresponding with Dr. Roholm for several years, I visited Copenhagen in 1948 with the express purpose and desire to meet with him again, only to learn of his untimely death three months prior to my arrival. His monograph and his original studies constitute a major contribution to our knowledge of the physiological effects of fluoride.

F. J. McClure

A REVIEW OF FLUORINE AND ITS PHYSIOLOGICAL EFFECTS

F. J. McClure

Physiological Reviews—July 1933

As early as 1670 it was known that glass is attacked by the fumes produced when fluorspar is treated with sulfuric acid. It was not until 1802, however, that Morichini used this reaction apparently for the first time, to test for the presence of these same "fumes" in a substance of animal origin. As a result of his studies he reported the presence of F in fossil teeth. Later (1805) Gay-Lussac and Berthollet found F in enamel of normal teeth and thus opened the question, which continues to this day, as to the extent and significance of the occurrence of F in teeth and other body substances. Sometime later F was found present in the ash of blood and of milk; in 1869 it was reported present in the brain; and in 1888 its discovery in the yolk and shell of the egg, brought forth the suggestion of an important physiological role. In 1912 Gautier and Clausmann, who have published the most extensive analytical results for F, presented their first work and have since reported results, which together with those of other investigators, indicate that F is widely distributed in soils and waters and in plant and animal substances.

The toxicity of fluorides to man became evident when the chemists Thenard and Davy, while attempting to prepare pure F, were made seriously ill by accidentally breathing the vapor of hydrofluoric acid, and when Louyet and Nickles lost their lives from the same cause. Such violent effects naturally occasioned concern as to the probable toxicity of F contained in drinking waters, in wines and beers, in phosphate baking powders, and in other foodstuffs in which F might occur naturally or, as was the case following the discovery of its antiseptic properties, might be present in the form of a preservative. Experimental evidence of the toxic effect of F on man and animals is, therefore, abundant in the literature. Interest in the physiological role of F was stimulated somewhat by the observation made by McCollum et al., on the effect of F on the teeth and by the work of Embden and his school as to its effect on muscle metabolism, an effect not unrelated to its action on certain enzymatic changes. A group of investigators in this country have studied the effects of ingested fluorides, for the sake of assessing the significance of the small amount of F which occurs naturally in rock phosphate and thus becomes a not inconsiderable part of the intake of animals fed rock phosphate as a calcium mineral supplement. During the past year evidence has been presented which indicates that F in drinking water may be the cause of a defect of human teeth known as mottled enamel. Most experiments planned, therefore, to study the physiological effects of F have had to do with the following problems: *a*, the distribution and purpose of F in the animal body; *b*, the effect of small quantities of ingested fluorides on food consumption, growth and reproduction; *c*, the relation of F to the development of teeth and bones, and *d*, the effect of fluorides on certain enzymatic changes related particularly to fat and carbohydrate metabolism.

DISTRIBUTION OF FLUORINE

Disagreement among analysts reporting quantitative results for F, wherever it occurs in very small amounts, may be attributed largely to difficulties involved in the analytical procedure. The notable chemical activity of F, as well as the volatile nature of many of its compounds, has contributed to these difficulties. Despite the fact that numerous methods have been devised and have been used, at least by their originators, it is not established that any F method now known is adequate for the quantitative analysis of plant and animal substances. The most promising methods for the purpose, however, are *a*, colorimetric methods (*1, 2, 3, 4, 5*); *b*, gravimetric methods based on the formation of CaF_2 (*6, 7*) and on the formation of fluorides of rare metals, as thorium fluoride ($ThF_4 \cdot 4H_2O$) (*8*), and lanthanum fluoride (LaF_3) (*9*), or *c*, methods which consist in passing volatilized SiF_4 into water and then determining, either volumetrically or gravimetrically, the amount of H_2SiF_6 formed (*10, 11, 12, 13, 14*).

a. In minerals, soils, and waters.—Fluorine occurs most abundantly in nature in the mineral fluorspar (CaF_2) and in cryolite, a double fluoride of aluminum and sodium. Minerals containing small amounts of F as biotite, tourmaline, muscovite, and apatite, distributed throughout the earth's crust, account for part of the F found present in soils. According to Steinkoenig (*15*), F in soils amounts to about 0.03 percent.

Jacob and Reynolds (*16*) have investigated the F content of various commercial grades and types of phosphate rock. The amounts of F found, vary from 2.62 percent in Tennessee phosphate "run of mine material" to 4.08 percent for "carefully selected lump rock." A "fertilizer grade" of the same rock contained 3.72 percent F, while a high grade washed and ground rock contained 3.89 percent. These values are similar to those obtained for samples of phosphate rock occurring in North Africa, Idaho, Wyoming, and Florida.

The presence of F in mineral, river and sea waters seems established. Carles (*17*) found mineral waters containing 0.001–0.018 gram F per liter. River water analyzed by Gautier and Clausmann (*18*) contained 0.02 to 0.60 mg. F per liter. Carnot (*19*) found 0.822 mg. F per liter of ocean water. Smith, Lantz and Smith (*20*) found that 3.8 to 7.15 mg. F per liter in drinking water caused mottled teeth in humans. Other waters not harmful showed 0.0–0.3 mg. F per liter. Churchill (*21*) reports from 2.0 to 13.7 mg. F per liter in drinking waters of localities where mottled enamel is endemic.

b. In plants and other foodstuffs.—Gautier and Clausmann (*22*), on examination of 63 plant substances, found an average of 2.65 mg. (0.00265 percent) F per 100 grams of fresh material. Apparently more F is present in leaves than in stems and there is more in the skin of fruit than in the pulp. According to these authors the F content of some edible substances (reported on the fresh basis) is as follows: corn meal 0.00117 percent; rye meal 0.00052 percent; kidney bean 0.00170 percent; cabbage 0.000088 percent; cauliflower 0.00021 percent; apple pulp 0.000034 percent; apple skin 0.00076 percent; strawberry 0.00012 percent; potato 0.000084 percent; tomato 0.00020 percent and buckwheat 0.00217 percent. The average for 32 representative edible food substances is 0.000573 percent F for the fresh material.

Woodman and Talbot (*23*) found "that the presence of a slight amount of F in malt liquors is the rule, rather than the exception," and "that the principal source of this fluoride is the malt itself." They set a maximum limit of permissible fluoride in malt liquors at 10 mg. per liter.

c. In bones and teeth.—In 1802 Morichini (cited by Gay-Lussac and Berthollet) (*24*) found F in fossil teeth and later (1805) he, as well as Gay-Lussac and Berthollet (*24*), was convinced of the presence of F in normal human teeth. In 1807 Berzelius (*25*) reported 1.0–2.0 percent F in bones but later found his results to be erroneous. It was believed generally, during the time of Berzelius, that fresh bone contains much less F than fossil bone. However Berzelius believed F not to be an essential constituent of normal bone.

Owing to the difficulties involved in the direct determination of F it became more and more the custom to report the F in bone by difference and thus Weiske (*26*) reported 3.8 percent F in the ash of chicken bone; and results as high 5.0–8.0 percent F in the whole bone, were recorded. The values obtained by Carnot (*27*), varying from 0.1–0.3 percent for bone ash, agree with those established by Zalesky (*28*).

In experiments conducted by Brandl and Tappeiner (*29*), the deposition of F compounds in the body following F feeding was demonstrated, but no F was found in the ash of normal bones. Gabriel (*30*) made a thorough study of the method of Fresenius, modified the method somewhat, and by means of numerous careful analyses of teeth and bones from various sources, obtained the low value of 0.05 percent, as the F content of normal tooth and bone ash. In a few samples of ox bone ash he found 0.10 percent F. Gabriel thought that his results might be taken as indicating that bones and teeth are entirely free of F.

Wrampelmeyer (*31*) found from 0.65 percent to 1.40 percent F respectively in sound and carious teeth of children and adults. Having found F in bone ash in amounts varying between 0.05 and 0.32 percent, Jodlbauer (*32*) regarded F as a constant constituent of bone tissue.

However, the gas volume method used by Jodlbauer, has been justly criticized by Gassman (*33*), who showed that warming bone ash with concentrated H_2SO_4 formed gaseous HCl as well as SiF_4, and that consequently volumetric measurements of gases thus formed did not indicate SiF_4 quantitatively. Gassman believed that normal bones do not contain F.

Among the more recent reports of the F content of bones and teeth there should be mentioned the results of Gautier and Clausmann (*34, 35*), Trebitsch (*36*), and Bethke, Kick, Edgington and Wilder (*37*). The results of Gautier and Clausmann for teeth and bones show the approximate values of 150 mg. of F per 100 gr. of dry dental enamel and 46 mg. per 100 grams of bone ash. The Meyer and Schulz (*9*) method was used by Trebitsch. His values for F in the dry substance of human bones are extremely high (0.29–0.59 percent). According to Bethke, et al., the ash of the femurs from two lots of pigs without added F in their ration contained 0.0409 percent and 0.0231 percent F respectively; representing 0.0251 percent and 0.0142 percent of F respectively for whole dry bone.

In summarizing the results which have been reported for bones and teeth, it is to be noted that Gautier and Clausmann obtained values for bone ash varying between 17.5 mg. and 123.0 mg. F per 100 grams of ash. Jodlbauer's results are high, due, no doubt, to errors in his method. His values vary between 50 mg. and 540 mg. F per 100 grams of ash, and average 191 mg. The values of Zalesky and Carnot are between 170 mg. and 310 mg. F per 100 grams of ash. Since normal bone contains approximately 60 percent of ash, the average values of Gautier and Clausmann represent about 0.0298 percent F in the whole bone. Bethke et al. report 0.0251 percent and 0.0142 percent for normal bone. The majority of the analytical results appearing in the literature may be regarded as indicating that normal bone contains between 0.01 percent and 0.03 percent F, though there is evidence that great variations in F content occur. It is to be recalled that Gabriel, Brandl and Tappeiner as well as Gassman, believed bones not to contain F.

The results of F determinations on teeth are more limited and perhaps more variable than those reported for bones. Gautier and Clausmann's results vary from 0.118 percent to 0.180 percent F in dental enamel on a dry basis. Carnot found dental enamel on the dry basis to contain 0.10 percent–0.30 percent F. It cannot be said that it is established that the F content of teeth is as high as these values indicate. According to some investigators there is less F in teeth than in bones, or as reported by Casares and Casares (*59*), who have recently perfected a qualitative test for F in bones and teeth, F does not exist in teeth. However the quantitative results for F which appear in the literature up to the present time indicate the presence of about 0.10–0.20 percent F in normal dental enamel.

d. In other animal organs and tissues.—Of the tissues examined by Gautier and Clausmann (*38*), the richest in F are the following: dental enamel, containing 118 mg. to 180 mg. of F (these values and those immediately following represent milligrams of F per 100 grams of dry substance); bone (diaphysis) 56.0 mg. to 87.0 mg.; epidermis 16.4 mg.; hair 6.1 mg. to 19.7 mg.; thymus 3.9 mg. to 11.1 mg.; testicle 1.2 mg. to 4.2 mg.; blood 2.3 mg. to 4.4 mg.; and hair 1.0 to 3.0 mg. Tissues poorest in F are cartilage, 0.4 mg. to 1.4 mg., and muscle, 0.04 mg. to 0.46 mg.

Apparently blood is high in F (*38, 39, 40*). According to Gautier and Clausmann (*38*), 100 grams of dry blood contain approximately 3.5 mg. of F, depending somewhat on the species. Brandl and Tappeiner (*29*) found 120 mg. NaF (54.27 mg. F) in 100 grams dry blood and 19 mg. NaF (8.59 mg. F) in 100 grams of fresh blood. Stuber and Lang (*41*) found values varying from 0.0 to 0.94 mg. F per 100 grams of fresh blood. In most cases the results which they obtained were thought to be abnormally high, due presumably to the presence of relatively large amounts of F in the drinking water.

Three samples of fresh cow's milk, as analyzed by Stuber and Lang, contained respectively 0.11 mg., 0.16 mg. and 0.20 mg. F per 100 grams. Gautier and Clausmann report 0.046 mg. and 0.051 mg. F for two samples of human milk, and 0.180 mg. F for cow's milk, per 100 grams of fresh milk.

SIGNIFICANCE OF THE OCCURRENCE OF FLUORINE IN ANIMAL ORGANS AND TISSUES

It is apparent that F is found in many of the organs and tissues of the body and may be excreted from the body in both the urine and feces. Schulz (*42*) first expressed the opinion that F is excreted through the urine combined with alkali. Tappeiner (*43*) fed a dog 0.5 gram NaF, and the urine of the two following days contained 0.124 gram of NaF. Another dog received 0.240 gram of NaF; 0.059 gram appearing in the urine of the two following days. Brandl and Tappeiner (*29*) fed varying daily amounts of F to a young growing dog for a period of about 21 months. The total NaF in the feed for the period amounted to 402.9 grams and in the feces and urine it amounted to a total of 330.5 grams; 72.6 grams of NaF apparently were deposited in the organism. The blood, muscles, liver, skin, bones, cartilages, and teeth were weighed and analyzed and a total of 64.64 grams of NaF reported present in the whole body. Gautier and Clausmann (*38*) estimate a daily excretion by man of 0.23 mg. F in the urine, and 0.80 mg. in the feces (a total of 1.03 mg.); F in the diet probably exceeds 1.0 mg. per day. To account for further loss of F from the body Gautier and Clausmann as well as Brandl and Tappeiner call attention to the accumulation of F in the hair, nails, and epidermis as a means of F excretion. It may be concluded from the foregoing facts that the metabolism of F is probably a normal function of the animal organism.

Gautier and Clausmann have been lead to believe that F plays a "necessary and specific role" in the animal body. Gautier (*44*) classifies the different tissues of the body into three groups, according to their F content. The first group is comprised of organs of assimilation and secretion, highly differentiated and having an active metabolism, but containing very small amounts of F. A second group of tissues comprises the bones, cartilage, tendons, etc., which contain F varying from 88.0 mg. (bone) to 4.5 mg. (cartilage) per 100 grams of dry matter. The third group comprises organs for "mechanical" protection or defense or for ornamentation, such as hair, feathers, nails, epidermis, etc. These tissues are rich in F, containing about 180 mg. per 100 grams of dry tissue. Fluorine in these tissues he regards as playing a secondary rôle associated with the hardness, the resistance, and to a certain degree, *"d'inalterabilite chemique"* of such tissue.

Calcium fluophosphates occur naturally and may represent a form in which F could occur in bones and teeth, since the formula for the calcium-phosphorus compound of bones and teeth may not be known (*45*). According to Trebitsch (*36*) it has been suggested as a result of Roentgen-analysis, that the hardest apatite, namely fluor-apatite, $3[Ca_3(PO_4)_2] \cdot CaF_2$ occurs in dental enamel and that the hardness of teeth depends on the presence of crystal masses of apatite. Thus there is a tendency to regard F as an essential constituent, particularly of the teeth. Recently there has been presented clinical as well as experimental evidence of the fact that mottled enamel, a defect of human teeth, may be an endemic imperfection caused by the action of F present in the drinking water. These and other experiments have demonstrated the fact that regardless of their F content, teeth are influenced structurally and presumably chemically by the presence of extremely small amounts of F in the diet. For this reason it appears that the intake of the element must be kept at an extremely low level. On the other hand no experimental evidence has demonstrated the indispensability of F or the specific effects of the complete withdrawal of F from the diet. Mere traces of F are known to exert a potent influence on the reactions of certain enzymes (as well as on tooth formation) and further investigation may reveal a specific role of F in certain chemical changes necessary for normal cell metabolism.

PHYSIOLOGICAL EFFECTS OF FLUORINE

a. Fluorine toxic to man and other animals.—Numerous cases are on record which show that the ingestion of F compounds beyond certain limits is fatal to man and other animals (*46, 47*). A clinical picture of the effect of the ingestion of small doses of fluorides is described by Tappeiner (*43*) and by Schulz (*42*). Tappeiner used dogs, rabbits, guinea pigs and cats as experimental animals. As high as 0.5 grams NaF per 100 grams body weight, was given internally and 0.15 gram was given by injection subcutaneously and intravenously. The following characteristic symptoms were observed: 1, a condition of drowsiness and weakness resulting from paralysis of the vaso-motor centers; 2, cramps which may attack a single organ or the entire body, and are epileptic in character. They are not so evident in cases of internal poisoning, although in many instances they dominate the poisoning effects; as nonreflex changes, they are independent of the disturbance of the breathing and circulation and proceed from the spinal cord and more highly centralized organs; 3, paralysis of the vasomotor centers; 4, acceleration and deepening of the breathing with paralysis following; 5, vomiting; 6, secretion of the salivary and tear glands, which is not stopped with atropin; 7, early rigor following death.

There is unmistakable evidence in the data reported by Goldemberg (*48*) that the injection of NaF lowers the basal metabolic rate of white rats. Goldemberg employed the method devised by Haldane for the indirect determination of heat production. The heat produced by the experimental animal was determined before and after the intraperitoneal injection of the fluoride solutions. Amounts of NaF varying from 15 to 18 mg. per kgm. of body weight lowered the heat production per kilogram per hour as much as 12 percent to 63 percent below the previous rate. The effects were noted usually 15 minutes to about 60 minutes after the injection.

Goldemberg (*49*) also observed a local ulcerous condition at the point of injection of fluorides, as well as toxic symptoms similar to those reported by Tappeiner (*43*) and Schulz (*42*).

The work of Cristiani and Gautier (*50, 51, 52, 53*), of Cristiani and Chausse (*54, 55*), and Cristiani (*56, 57*) has generally confirmed the results of earlier experiments as regards the symptoms of F poisoning. This group of investigators believe there is an absorption of F by the vegetation in the vicinity of aluminum plants which give off waste gases containing F. Apparently the herbivorous animals of the vicinity are in some way affected by the vegetation which they consume. Cristiani and Gautier (*51*) claim to have reproduced toxic effects characteristic of fluorides by feeding the suspected forage to guinea pigs. Sette (*58*) regards these toxic symptoms as a general acid intoxication, which might result from HNO_3 and H_2SO_4 vapors contained in the air.

It becomes obvious on inspection of the data on the subject (*61, 62, 63, 64, 65, 66, 67, 68, 69*) that the form of F used, the method and length of time of administration, as well as individual susceptibilities, have varied the toxic effects to such an extent that a comparison of the data obtained is scarcely warranted. However, there is evidence that fluosilicates are more toxic than either NaF or CaF_2 and that NaF is more toxic than CaF_2. Other constituents of the ratio may perhaps have affected the results in numerous instances. The level of toxicity of F in any form, for man particularly, is of some importance because of the fact that the antiseptic property of fluorides has occasioned their use as a preservative in wines and beers, tomato catsups, various plant juices, etc. There is still some controversy as to the desirability of their use for this purpose and it would appear that more work is needed to determine at what level F may be used in this connection without its becoming toxic. As was mentioned, drinking waters containing quantities of F varying from 3.8 to 7.15 mg. per liter may affect, detrimentally, normal tooth development and may therefore, become the source of a F intake which exceeds the hygienic level.

b. Effect of fluorine on development of bones and teeth: Effect of amount of fluorine present.—There is evidence that the F of bones and teeth, as well as of other body organs and tissues, may be increased by an increase in the F intake. Sonntag (*59*) found that whereas normal teeth and bones contain about 0.3 percent F, the teeth of animals fed NaF contain 1.73 percent F and the bones 1.29 percent F. Christiani and Gautier (*50, 51, 52*) believe that F tends to accumulate in the bony tissue producing a condition resembling osteomalacia. According to Cristiani (*56*) the deposition of F in bone is a very reliable index of F toxicity.

Bethke et al. (*37*) found that the ash of femurs of two groups of pigs receiving limestone or steamed bone meal as a Ca mineral supplement, contained 0.0409 and 0.0231 percent F respectively, while bone ash of femurs from lots of pigs receiving varying amounts of F in the form of NaF or rock phosphate contained 0.528 percent, 0.7935 percent, 1.1077 percent, 0.5937 percent, 1.0934 percent, and 1.0369 percent F.

The bones of a young growing dog fed NaF in small amounts by Brandl and Tappeiner (*29*) for a period of about 21 months, were examined microscopically. These investigators found deposited in the bones, vivid, glistening crystals which they regarded as crystalline compound similar to fluorite (CaF_2). They believed that soluble fluoride salts in the feed are deposited in the bones in considerable amounts but they were unable to find any crystals in the teeth. An increased brittleness of some of the bones was noted also.

Structural changes.—Structural changes in teeth following the ingestion of small amounts of F, were observed by McCollum, Simmonds, Becker and Bunting (*65*). Sodium fluoride to the extent of 0.0226 percent (0.0101 percent F) was included in an otherwise normal diet of rats. It was observed that the rats receiving the added F developed incisors which were abnormal in color, lacking the characteristic orange tint on the anterior surface. There were striking changes in the shape of the teeth. The upper incissors grew backward into a circle, while the corresponding lower incissors were shortened.

These changes appear to be due to an alteration in the structure and hardness of the teeth which, according to McCollum et al., resulted finally in "the lack of the natural wear which the incisors of the rat under normal conditions exert on each other in occlusion." It is known that if for any reason this incisal reduction by wear does not take place, the incisors undergo a marked overgrowth quite similar to the overgrowth occurring in the teeth of rats fed F. Apparently the lower incisors, due to their inferior quality, are promptly fractured while the upper incisors, lacking the necessary wear, grow to an abnormal length. The authors state that the manifest inferior quality of these teeth indicates "a retrograde disturbance of tooth development, rather than a stimulation to overactivity." Chaneles (*70*) made a microscopic study of the teeth of rats fed fluorides and describes, in detail, changes in the structure and arrangement of the cells.

Changes similar to those observed by McCollum, in teeth of rats, were produced by Tolle and Maynard (*72*) by feeding rock phosphate, and by investigators at the Arkansas Agricultural Experiment Station (*73*), who fed NaF and CaF_2. Bergara (*74*) observed such changes by feeding NaF, and stated also that the incisor teeth lost their reddish yellow color, finally showing dark bands. At first the teeth seemed more opaque and lighter.

Results obtained at the Michigan Agricultural Experiment Station (*75, 76*) have demonstrated an effect of F on teeth of dairy cattle by the feeding of either $Na_2SiF_6 \cdot 2H_2O$ or rock phosphate. The mandibles of these cattle were much thicker and the surfaces were rougher than those of normal cattle. McClure and Mitchell (*68*) again demonstrated that "a level of 0.03 percent and 0.06 percent fluorine in the form of sodium fluoride or calcium fluoride brought about the characteristic abnormalities," in rats. However, McClure and Mitchell (*69*), and Mitchell (*77*), observed no outward changes in the teeth of swine as a result of the presence of fluorides in the ration.

Effect on other constitutents.—Experiments have been reported which indicate that a change in structure and in the content of elements other than F may occur in the bones of animals fed fluorides. McClure and Mitcheell (*68*) found "that sodium fluoride at levels of approximately 0.03 percent and 0.06 percent fluorine caused a consistent increase, averaging 1.30 percent, in the ash content of the bones" and "a statistically significant, though not as consistent a decrease, in the calcium content of the ash, averaging 1.05 percent. The phosphorus content of the ash was not significantly affected, but the ratio of calcium to phosphorus in the ash was depressed." There was a tendency for CaF_2 at the same levels of F to increase the ash in the bones and to depress the Ca in the ash of the femurs but not in the ash of the humeri.

Contrary to the above findings as applied to swine nutrition, Bethke and coworkers (*37*) found no changes in the total ash, or in the Ca and P content of the ash following additions of fluorides to the ration of young growing pigs.

Schwyzer (*62*) compared the total Cl content of fresh bone of normal dogs with the Cl in bones from two dogs fed fluorides. His results do not include a sufficient number of individuals to warrant any conclusions regarding the effect of F. Likewise the report of the Ca content and specific gravity (*61*) of the bones is incomplete and cannot be interpreted as indicating any differences due to F.

Mitchell (*77*) studied the composition of the humeri and scapulas of pigs as affected by CaF_2 and by rock phosphate additions to the ration. As regards the effects of feeding CaF_2 in these experiments, the most that may be said is that there is rather strong evidence of a decrease in the Ca and P in the ash of the humeri. This is consistent with the finding of McClure and Mitchell as regards the Ca and P of the ash of bones of rats fed fluorides. Bethke, it is recalled, observed no such changes in the bones of pigs. Mitchell's results do not warrant conclusions as regards Ca in the water-free and fat-free bones.

Mottled enamel.—A defect in human teeth known as mottled enamel is endemic in certain states in the United States, in South America, China, Holland, Spain, Italy, Mexico, South Africa, Bahama and the Cape Verde Islands (*78*). The cause of this abnormality has been attributed to the water supply, particularly by McKay (*78*). Recently Smith, Lantz and Smith (*20*) presented the first experimental evidence that F contained in drinking water is probably the causal factor. Churchill (*21*) has found F present in waters from localities affected with mottled enamel. Smith, Lantz and Smith investigated endemic mottled enamel occurring in St. David, Arizona. Examination was made of the teeth of children in elementary grades and high school, and subsequently the occupants of nearly every home in the community were examined. According to these investigators "every child exposed to the environmental conditions of this community during the years of growth of the enamel of the permanent teeth is certain to have mottled enamel." The fact that the water supply is the source of the trouble appears to have been proved by the production of a similiar condition in the enamel of experimental rats by giving the rats St. David water to drink or by giving food in which the residue from St. David water had been incorporated. "The mottling of the enamel thus produced has been found to be identical with that resulting from the inclusion of sodium fluoride in the ration." Quantitative analysis of St. David drinking waters revealed the presence of 3.8 to 7.15 mg. of F per liter as compared with 0 to 0.3 mg. in waters from nonendemic communities.

Mottled enamel in human teeth is characterized by chalky white patches distributed over the surface; frequently the enamel is pitted and corroded. There is an absence of normal transluscency, the teeth showing a dead white unglazed appearance. Histological examinations according to Black (*79*) and Leon (*80*) indicate imperfect calcification particularly of the enamel rods themselves. The intercementing material which is normally present between the enamel rods is lacking.

It may be concluded that fluorides produce pronounced and characteristic structural changes in teeth. There is evidence that bones may also be affected structurally by fluorides. Apparently F is present in excessive amounts in the bones and teeth of animals fed fluorides. There is evidence that the ash, Ca and P content of bones may be affected detrimentally by the ingestion of small amounts of fluorides. From the results of their experiments with rats, McClure and Mitchell concluded "that fluorine, especially when consumed in the more soluble form of the sodium salt, may cause the deposition of an apparently abnormal constituent in the bones, or an abnormal deposition of a noncalcium constitutent, possibly a fluoride of a mineral other than calcium, as evidenced by an increase in percentage of ash in the bones above the normal. There is also an interference with the deposition of calcium in the bones brought about by feeding fluorine, a result in agreement with the calcium balance studies previously described." At what appear to be extremely low levels of F intake, these defects may be manifested in the growth and outward appearance of the teeth.

c. Effect of ingestion of small amounts of fluorine on food consumption, growth, and reproduction.—Chronic F intoxication in albino rats as studied by Sollman, Schettler and Wetzel (*63*), included the feeding of NaF, NaCl, "phosphate rock," $Ca_3(PO_4)_2$ (precipitated) and $CaCO_3$ (precipitated). Doses of pure NaF, ranging from 0.0015 mg. to 151.0 mg. per kg. of body weight daily, were added to an otherwise complete ration, and the effect on food consumption and growth was observed. Generally, the results obtained show a parallelism between food intake and growth. In three instances, however, the food consumption was normal or above, whereas the rate of growth was below normal. The amount of F in the rations in these cases varied from 0.018 to 0.0452 percent. These results may be regarded as indicating an effect of F at these levels of intake on the rate of growth of rats. An amount of F equivalent to 0.103 percent of the ration proved fatal to seven rats within 11 weeks. Six rats received a ration containing 0.045 percent F, along with a normal ration, both fed *ad libitum*. Three of these rats died by the end of the fifth week. Since the results show that all the fatalities occurred on the higher level of F feeding, it may be concluded that F was the causal factor.

Schulz and Lamb (*66*) added NaF to a ration which had proved adequate for normal growth and reproduction of rats, in amounts representing 0.0226, 0.0452, 0.0678, and 0.1130 percent F. It is evident that the higher amount of F is definitely toxic, but it is difficult to interpret the effects of the lower amounts in the absence of data concerning the food intake and the rate of growth of the individual rats. Regarding the effect on reproduction the authors state, "It appears that an unfavorable effect on reproduction begins at a level of about 0.0250 percent NaF (0.0102 percent F) in the ration." Confirming this observation as regards reproduction in rats, Del Castillo (*81*) and Chaneles (*70*) report that fluorides interfere with the estrual cycle of rats.

Rats fed daily doses of 0.002–0.003 gram of NaF by Goldemberg (*49*) showed greatly retarded growth. This investigator chooses to call the effect a *cretinisme fluorique*, even suggesting that endemic cretinism may be due to F contained in the soil, air, food and water, of affected regions. Goldemberg's work would appear to lack proper control and his assumptions need further experimental confirmation, particularly as regards endemic cretinism and also endemic goiter. (His observations with regard to the effect of F on the thyroid gland will be mentioned later.)

According to investigators at the Iowa Agricultural Experiment Station (*82, 83*), the results of studies on the effect of fluorides in the ration upon the growth and reproduction of albino rats justify the following conclusions: "the toxic effects produced by sodium fluoride vary directly with the amount of calcium salts in the ration;" "the toxic effects of calcium fluoride are much less marked than those of sodium fluoride." "Normal growth and reproduction proceeded even when the concentration of CaF_2 was increased to 0.25 percent (0.113 percent F) in a ration composed of corn, tankage, linseed oil meal, bone meal, and salt. In more highly purified rations which contained wheat, limestone, and butterfat, growth and reproduction failed when the concentration of CaF_2 was at a lower level."

McClure and Mitchell (68) studied primarily the effect of F on growth, bone and tooth development and the utilization of Ca by the experimental animals. Young growing rats were fed NaF by the paired feeding method, in an otherwise normal ration, in amounts equivalent to approximately 0.01, 0.03 and 0.06 percent F. Two levels of CaF_2 providing about 0.03 percent and 0.06 percent F were also fed to growing rats. Inspection of the growth curves as well as a statistical analysis of the growth data warrants the conclusion "That calcium fluoride as well as sodium fluoride at levels of 0.0313 percent and 0.0623 per cent fluorine in the ration inhibit growth, entirely aside from any effect on food consumption." "The higher level of sodium fluoride appears to have lowered the food consumption of the rats also, but the corresponding level of calcium fluoride had no apparent influence on food consumption."

In dairy rations.—In an attempt to duplicate toxic effects obtained by feeding raw rock phosphate to dairy cattle, Taylor (*67*) at the Michigan Agricultural Experiment Station, has fed several dairy cows varying amounts of F in the form of calcium fluosilicate, $CaSiF_6 \cdot 2H_2O$. One animal fed F at a level of 0.125 percent of the dry matter of the ration, went off feed at once and "refused to eat either its roughage or grain mixture in any appreciable amounts for a period of 3 weeks." At this time the amount of F was reduced to 0.4 gram daily, and the ration was properly consumed. Five grams of $CaSiF_6 \cdot 2H_2O$ (2.28 grams of F) fed daily to one cow caused no deleterious effects until after the cow freshened, when there was a rapid decline in weight and a loss of appetite. The above response to F feeding has been repeated in other dairy cows by the same investigator, and the conclusion "that fluorine is detrimental to health and general condition of dairy animals" at the levels fed seems justified.

In swine rations.—The experiments of Bethke, Kick, Edgington and Wilder (*37*) show that F in the form of NaF, when added to the ration of growing swine, among other effects lowers the food consumption. Since the data show that the average daily gain among the different lots of pigs varies directly with the food consumption, there is difficulty in assessing the role of F in the growth response observed in these experiments.

Concurrent with their above mentioned experiments with rats, McClure and Mitchell studied the effect of CaF_2 and phosphate rock on the calcium retention of young growing pigs (*69*). The results of feeding fluorides to three pigs at levels of about 0.013, 0.017, and 0.026 percent F (0.0032, 0.0035, and 0.0055 gram F per kg. of body weight respectively), are regarded as having demonstrated a detrimental effect upon the food consumption and growth of these pigs.

There is unmistakable evidence that food consumption is less and the rate of growth is retarded, due to the ingestion of small quantities of fluorides. The evidence indicates that levels of F as low as 0.03 percent F in the ration may inhibit growth of rats. It is possible that the form of F as well as other constituents of the ration affect the toxic level. Apparently the dairy cow, as indicated by the general nutritive state as well as by the refusal to consume food, is particularly susceptible to the effects of very small amounts of F. The evidence is less striking with regard to the response of swine. However, the experiments with rock phosphate in which swine have been used as the experimental animals, indicate toxic disturbances, which in the present state of our knowledge, are generally attributed to F contained in the rock phosphate. There is evidence to indicate that 0.01 percent F in the ration may interfere with normal reproduction in rats.

d. Effect of fluorine on enzymatic changes: Reactions of lipase.—An inhibitory effect on enzymes characteristic of antiseptics in general is a property of ordinary inorganic fluorides. Experimental evidence has established the fact that there is also a specific influence of fluorides on certain enzymatic changes associated particularly with carbohydrates and fats. Thus the results of a systematic study conducted by Kastle and Loevenhart (*84*) on the effect of antiseptics on the reactions of pancreatic and liver extracts, revealed a harmful effect of most substances studied and also a particularly remarkable destructive action of NaF on the reactions of lipase. Subsequently Loevenhart and Peirce (*85*) investigated among other substances, the halides of Na and K; the chlorides of Ca, Cd, Ba and Mn; $NaNO_3$ and KNO_3; Na_2HPO_4; K_2CrO_4; and NH_4CNS, but none of these substances showed an inhibitory effect comparable to the action of F. Dilutions of NaF as low as 1:15,000,000 may inhibit the action of lipase on ethyl acetate as much as 50 percent. With ascending order of the fatty acid series the inhibition continues to decrease. Leake et al. (*86*) have obtained evidence that NaF also inhibits the action of this enzyme *in vivo*.

As regards the mechanism of this fluoride effect Amberg and Loevenhart (*87*) state that the facts indicate "that the fluoride probably acts by retarding the formation or decomposition of some intermediate product formed in the hydrolysis of the ester by the enzyme." That the action of NaF may be explained as a monomolecular combination of the enzyme with part of the NaF is the conclusion of Peirce (*88*) and of Murray (*89*). Rothschild (*90*) on the other hand believe that F by no means reacts with the free enzyme, though this does not mean that the effect is nonspecific. He suggests the possibility of a combination of F with the enzyme-substrate complex.

On carbohydrate enzymes.—Certain phases of the enzymatic breakdown of carbohydrate are particularly sensitive to an effect of the fluoride ion. The activity of the amylase of the potato as measured by its ability to produce invert sugar is increased by solutions of NaF up to 1/1.75 molar concentration (*91*). A study of the effect of oxalate (*92*), and fluoride (*121*), on glycolysis in blood, has revealed a strongly inhibitory effect. Lang and Lang (*93*) found that pancreatic diastase, as contained in an extract of beef pancreas, formed less maltose and a relatively greater amount of glucose in the presence of fluoride. It appeared to these authors that, while the total diastatic effect was inhibited by fluoride due to a decrease in breakdown of starch to maltose, there was a stimulating effect of fluoride on the maltose to glucose change. The work of Clifford (*94*) indicates an effect of fluorides on the enzymatic decomposition of starch. Clifford determined the time required for the achromatic point to appear in a 0.5 percent solution of starch treated with 2.0 cc. of saliva and various salt solutions. She reports that the Cl ion accelerates the reaction, the Br ion is inert, while the reactions of the fluorides and iodides are specific effects of the salts studied, i.e., NaF is inert while KF and NH_4F show a marked slowing effect.

Glycolysis in muscle.—Information as to the action of fluorides on glycolysis has been extended largely by the research conducted by Embden and his school (*95, 96, 97, 98*), concerning the influence exerted by ions upon the chemical changes associated with muscle metabolism. It became evident after certain experiments, that tartrate and fluoride inhibit or even reverse the glycogen breakdown changes of muscle. Of these two ions, fluoride is most potent and has received the greatest attention from the standpoint of the nature of the fluoride effect as well as for the elucidation of the nature of the chemical changes involved in muscle metabolism.

The early work of Embden and coworkers drew attention to the fact that F decreased the amount of free phosphoric acid formed in muscle pulps suspended in buffer solutions. It was supposed that the formation of a phosphate ester, described as lactacidogen, caused this decrease. The evidence indicated that the action of F might be a synthetic one, as for example Embden and Haymann found, with the disappearance of the free phosphoric acid, a definite formation of diphosphoric ester. This synthetic action can be increased by addition of glycogen to the fluoride solution (*99, 100*). Larger quantities of added phosphate may be synthesized if sufficient glycogen is added (*101, 102*).

Recent research, generally, has not confirmed Embden's original idea of the nature of the synthetic effect of fluoride. Thus Eggleton and Eggleton (*103*), having studied the effect of the fluoride ion as discovered by Embden and Lehnartz, state, "The 'synthesis' observed by these authors was in the main a conversion of phosphagen-phosphorus (which they estimated as inorganic P) into some acid-stable phosphoric ester. Where phosphagen is present some true synthesis can occur, however, for added inorganic phosphate disappears to a small extent under the action of fluoride." (Eggleton (*104*) has recently presented a review of the role of phosphorus in the chemical mechanism of muscular contraction, which includes a discussion of the developments of this phase of the subject.)

It is known that fluorides do not prevent a decrease in glycogen of muscle pulp undergoing fermentative changes but do prevent the formation of lactic acid (*105, 106, 107*). Fluorides also inhibit alcoholic fermentation by yeast (*108*), an observation which is not surprising, in view of the many analogies between lactic acid fermentation in muscle extract and alcoholic fermentation (a notable example of this similarity is the occurrence of practically the same hexosephosphates). Lipmann (*108*) sought to determine whether this inhibitory effect of F might not be due to a retarding of the hydrolysis of hexose-phosphorus-acid-ester, a compound which is formed in the enzymatic decomposition of glycogen. Meyerhof (*109*) as well as Lipmann (*110*), obtained evidence that the accumulation of hexose-diphosphoric acid, as observed by Embden under the influence of fluoride, is due to the inhibition of the dissociation of the intermediary-formed phosphoric-acid-ester, which in the absence of lactic acid formation goes over into a more enzyme-resistant form, i.e., hexose-diphosphoric acid. The strong inhibition is due to the position of F in the lyotropic series. The decrease in lactic acid formation is due to the inhibition of the hexose-phosphate breakdown. Lipmann (*110*), after studying carbohydrate fermentation by yeast as inhibited by fluorides, concluded that the inhibition is reversible, that the extent of the inhibition for different fluoride concentrations is in agreement with the law of mass action, and that the inhibition is greater with increased acidity. These properties are similar to those observed in connection with fluoride inhibition of lipase and agree with the assumption that F forms an easily dissociated compound with the enzyme, thus preventing the normal dissociation of hexosephosphate. Confirming this explanation is the fact shown by Lipmann (*110*), that the hydrolysis of glycerophosphoric acid, hexose diphosphoric acid and hexose monophosphoric acid is inhibited only slightly less by F than is lactic acid formation.

Lipmann (*110*) presents some evidence which indicates the formation of a complex compound of iron with F. Support for this view comes from a comparison of the above reactions with the dissociation curve of fluoro-methemoglobin.

Dickens and Simer (*111*) have confirmed the essential facts brought out by Lipmann and conclude in part "that fluoride forms an inactive compound with some substance essential for glycolysis. This reaction follows the ordinary laws of equilibria." Lohmann (*112*), in a recent contribution to the subject, states that the effect of fluorides, oxalates and citrates in lactic acid forming reactions of muscles, consists of the inhibition of the splitting off of phosphoric acid. As a result of this condition there is a change of first-formed (primary) hexose phosphoric acid ester into a difficultly hydrolysable ester.

Pepsin and urease.—Few striking effects of F on proteolytic enzymes have been demonstrated. Thus the digestion of white of egg, blood fibrin and protein of cows milk by pepsin and trypsin was found uninfluenced by the presence of fluorides (*113*). Von Groer (*114*) obtained some evidence that the gelatinase of Bacillus prodigiosus was retarded in its activity by the presence of a 1 percent solution of NaF. There is unmistakable evidence, however, presented by Clifford (*115, 116*), that the clotting time of milk under the influence of pepsin is retarded by fluorides. Inhibition may also be brought about by iodides but the reaction is hastened by chlorides and bromides. The halides of Na, K and NH_4 were studied by Clifford.

Jacoby (*117*) first observed that F inhibits the reaction of urease. Subsequent research (*118, 119*) by this investigator has brought out the following facts regarding the urease effect: *a*, F is most effective at optimum conditions for enzyme reactions; *b*, it is most active against urease in acid solution; *c*, the effect is more pronounced in buffered solutions on account of the reaction being influenced by the ammonia formed in the hydrolysis; *d*, the F effect cannot be duplicated with oxalic and citric acids; *e*, iodine salts are also very active against urease.

e. Additional body tissues and functions affected by fluorine.—Attempts to demonstrate an effect of F on the composition of various tissues (not including bones and teeth), other than an increase in the F content, have been generally unsuccessful. Trebitsch (*36*), as well as Brandl and Tappeiner (*29*) have reported the deposition of abnormal amounts of F in various body tissues by continuous small additions of F to the ration. Cristiani and Gautier (*50*) fed varying amounts (0.1–1.0 gram daily) of NaF to a dog for a period of about 21½ months, and there resulted what appear to be abnormal amounts of F deposited in various tissues. Schwyzer (*62*) reports an effect of F on some of the form elements of blood. Whereas 55–60 percent of the leucocytes of the blood of a normal rabbit are multinuclear, in the blood of rabbits fed F these values range from 10–34 percent. He believes these changes in the leucocytes are due to irritation or inflammation of the bone marrow. He noted a partial change of the yellow bone marrow into red.

On calcium metabolism.—Gerschmann (*120*) reports that the injection of 30 mg. NaF per kgm. body weight reduces blood Ca in 24 hrs. It cannot be said that Schwyzer (*62*) was successful in showing an effect of F on Ca metabolism. Direct experimental evidence of an effect of ingested F compounds on Ca metabolism was obtained by McClure and Mitchell (*68, 69*). These investigators saw fit to conclude that "the calcium balance of the rats was not affected by either sodium fluoride or calcium fluoride at levels of 0.0106 percent and 0.0313 percent F in the ration. The results indicate with a probability equal to practical certainty, that a level of 0.0623 percent of fluorine in the form of either calcium fluoride or sodium fluoride, lowered the percentage of calcium that otherwise would have been retained by the experimental rats" (*68*). The results of analyses show definitely a depression of the Ca content of the ash of bones of rats fed NaF and therefore may be regarded as further evidence of an effect of F on Ca metabolism.

Clotting of blood: It has been established that there is a small amount of F present in normal blood and that ingested fluorides may increase significantly this amount. Stuber and Lang (*121*) observed a number of cases of haemophilic patients with amounts of blood F so abnormally high, that they were lead to suspect F as being a causal factor. In two patients they found clotting times of 150 and 180 minutes associated with 0.0029 percent and 0.0039 percent F, respectively, present in the blood. It was recalled by these investigators that normal goose and rabbit blood clots slowly, and according to Stuber and Lang (*121*) relatively large amounts of F occur in the blood of these two species. Cat blood and dog blood clots rapidly and was found free of F by Stuber and Lang.

Continuing their observations relative to the blood clotting process, these investigators discovered that the blood of normal individuals residing in the town of Freiburg, Germany, would clot in from 6 to 9 minutes, whereas in the region of Kiel, the clotting time varied from 10 to 17 minutes. Their results of analysis for F in the blood of Kiel "patients" show much variation. In the absence of a report of the F content of blood from Freiburg residents it is impossible to assess the significance of their data. Apparently in numerous instances a large amount of F has accompanied a long clotting period. Analysis of Kiel tapwater revealed a relatively high content of fluorine as compared with Freiburg water, which contained no fluorine. Lang (*122*), however, found that ingested organic fluorine compounds had no effect on blood fluorine or coagulation time.

According to Stuber and Lang the rate of glycolysis in the blood of Kiel patients was less, due to the presence of fluoride. These investigators are of the opinion that a retarding of glycolysis may affect indirectly the clotting power.

Contrary to the results of Stuber and Lang, Schwyzer (*62*) observed a very high degree of coagulability of blood of rabbits, pigeons and dogs which were fed F. Whereas the coagulation of the total blood of a normal rabbit in an open vessel required from 6–8 minutes, the coagulation time in the case of fluorine-rabbits was 4 minutes in one instance and 2½ minutes in another. Blood in a capillary coagulated in 5 minutes for a normal rabbit and in 50 seconds, 1½ minutes, 3 minutes, 3½ minutes, and 2¾ minutes in the case of six rabbits fed fluoride.

On muscular irritability.—A form of toxicity of F directly ascribable to its ability to precipitate calcium is described by J. Loeb (*123*). Loeb put a fresh muscle (gastrocnemius) of a frog into dilute solutions of NaF, Na_2CO_3, Na_2HPO_4, sodium oxalate, sodium citrate and sodium tartrate; under this condition the muscle as a rule shows no reaction. When taken out of the salt solutions into the air, CO_2, oil, sugar solutions, etc., it begins to contract powerfully. If the nerve alone (without the muscle) is put into one of these salt solutions "the muscle begins to twitch in about 5 minutes and finally goes into tetanus." While Loeb believes that the diminution of Ca-ions is the cause of the effect of NaF and similar solutions, he considers it possible "that these solutions may have other effects which play a role in these phenomena." This form of F irritability has not received further investigation except as it is related to a disturbance of the normal ratio of salts in the muscle (*124, 125*).

Effect on thyroid glands.—Several reports appear in the literature to the effect that fluorine causes an enlargement of the thyroid gland. Maumeme (*126*) observed a swelling in the neck of a dog fed fluoride. His findings were not confirmed by other investigators.

Goldemberg (*49*) goes so far as to suggest that excess fluorine in the diet may be a cause of endemic goiter. He claims that by the ingestion or injection of 2 to 3 mg. of fluorine per day for 6 months the thyroid gland in white rats may be increased five to six times in volume. Cristiani (*127*) studied the condition of the thyroid glands of animals which had died of chronic fluorine poisoning. In the case of 14 such animals (guinea pigs) more or less marked lesions were observed in the thyroid glands. The alterations consisted essentially of a proliferation of the parenchymatous tissue, less rarely of the interstitial tissue.

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PHYSIOLOGIC EFFECTS OF FLUORIDE AS RELATED TO WATER FLUORIDATION

F. J. McClure and I. Zipkin

Probably no public health measure has been more thoroughly evaluated prior to its use by the public than the fluoridation of drinking water. A fluoridated communal water supply containing 1.0 p.p.m. of fluoride has proven to be remarkably effective for the partial control of dental caries. Fluoridated water is likewise free of harmful physiologic effects on those who use the water. The discovery of this highly beneficial relation of fluoride in drinking water to dental caries and its rapid adaptation to public use is without doubt the most significant development in the history of preventive dentistry.

Acceptance of fluoridated water by a community as a whole frequently has been accompanied by a great deal of controversy. Most of the reluctance to accept fluoridated water can be ascribed to the widely known acute toxicity of fluoride. The effects of an excessive quantity of fluoride, however, are not in any way a deterrent to its use in fluoridated drinking water. It is the purpose of this discussion to document briefly the major evidence and observations which permit the use of fluoridated water by large population groups without hazard to the public health.

Prior to the association of fluoride in drinking water with dental health, harmful physiologic effects of excess fluoride had been observed in several areas of exposure of both man and domestic animals to fluoride. An industrial process in which a dust-borne fluoride mineral (i.e., cryolite) was encountered by the workmen, fluoride in the gaseous effluent of certain industrial plants, as well as an excess of fluoride in the mineral nutrition of domestic animals, all have created major fluoride health problems. An elevated fluoride content of drinking water became a public health problem when it was found to be the cause of mottled enamel and when excessive amounts of fluoride in drinking water caused disabling skeletal defects. Much of the basic research on the chronic physiologic

Table 1.—*Summary of estimated daily intake of fluoride from food and from drinking water (drinking water containing 1.0 p.p.m. fluoride and dry substance of food containing 0.1 to 1.0 p.p.m. fluoride)*

AGE	BODY WEIGHT	DAILY FLUORIDE INTAKE			
		FROM DRINKING WATER	FROM FOOD	ACTUAL DAILY INTAKE	DAILY TOTAL RATE
yr.	kg.	mg.	mg.	mg.	mg./kg. body weight
1 to 3	8 to 16	0.390–0.560	0.027–0.265	0.417–0.825	0.026–0.103
4 to 6	13 to 24	0.520–0.745	0.036–0.360	0.556–1.105	0.023–0.085
7 to 9	16 to 35	0.650–0.930	0.045–0.450	0.695–1.380	0.020–0.068
10 to 12	25 to 54	0.810–1.165	0.056–0.560	0.866–1.725	0.016–0.069

effects of fluoride was motivated by these several areas of fluoride exposure.

For precision and quantity of the data on the physiologic effects of chronic fluoride toxicosis, the experimental results on domestic and laboratory animals exceed all other sources of information. However, because the absolute quantities of fluoride studied were often relatively large, an unequivocal application of these research data to water fluoridation is frequently not justified. Nonetheless, unquestionable evidence as to the organs and tissues of the body which are particularly sensitive to excess fluoride was made available through this extensive research preceding water fluoridation. The solution of problems connected with the public health aspects of water fluoridation was inestimably benefited by this prior information on the physiologic effects of fluoride.

SOURCES OF FLUORIDE

As a general rule the daily consumption of drinking water containing 1.00 p.p.m. or more fluoride is the major source of the total fluoride in the average daily diet. With estimates of the average intake of drinking water by adults varying from 1200 to 1500 cc. daily, and with water fluoridated at the optimal level of 1.0 p.p.m. fluoride, upwards of 1.2 to 1.5 mg. of water-borne fluoride will be consumed daily. Add to this an approximate 0.2 to 0.3 mg. of fluoride in the daily food, and the total daily fluoride intake amounts to 1.4 to 1.8 mg. While the great majority of ordinary foods as consumed contain approximately 0.2 to 0.3 p.p.m. or less of fluoride, notable exceptions are tea and sea foods. Better grades of tea may contain 75 to 100 p.p.m. of fluoride—a cup of good grade tea contains about 0.1 mg. of fluoride. Fresh and canned fish may contain from 2.0 to 12.0 mg. of fluoride per pound. Milk contains about 0.1 to 0.3 p.p.m. of fluoride and the level remains practically the same regardless of the dairy cows' fluoride intake. An excessive fluoride exposure may increase slightly the fluoride in cows' milk.

The quantities of the total daily fluoride provided by fluoridated water and the food are, therefore, very small indeed. This factor of quantity of fluoride ingested in the use of fluoridated drinking water is extremely important. It is all too often lost sight of in our attempts to resolve the health safety of water fluoridation. The following tabulations give some indication of the quantities of fluoride ingested by children when the drinking water contains 1.0 p.p.m. fluoride.

From the available data it appears that food-borne fluoride as well as water-borne fluoride is largely available for the body's assimilation. It should be emphasized also that there is no difference in the availability and metabolism of fluoride present as a natural constitutent of many drinking waters and fluoride added in fluoridated drinking water. It may be of interest to mention that the fluoridation of drinking water is accomplished mainly by the controlled addition of sodium fluoride or sodium fluosilicate to the water. Recently, progress has been made on the use of a mixture of calcium fluoride and alum.

FLUORIDE ELIMINATION

The most characteristic feature of fluoride biochemistry in human exposure is its accumulation in skeletal and dental tissues. It is for this reason more than any other that prolonged periods of excessive fluoride ingestion will become incompatible with physiologic safety, unless this ingested fluoride is eliminated from the body. Resolution of problems of cumulative toxic skeletal fluorosis have followed several lines of study. Obviously a foremost consideration was the elimination of fluoride from the body. Related to the fluoride content of drinking water, a comprehensive survey of urinary fluoride excretion was made prior to water fluoridation. Upwards of 90 percent of the fluoride ingested in drinking water that naturally contained up to 4.5 p.p.m. of fluoride was found to be excreted in the urine. A remarkably close correlation exists between the fluoride concentration of urine and the fluoride content of drinking water.

The relation between fluoride in drinking water and the urinary fluoride excretion applies equally to a natural fluoride water and fluoridated water. Thus, a survey has been made of the fluoride in the urine of individuals of different ages who were drinking water fluoridated to approximately 1.0 p.p.m. with sodium fluosilicate (Montgomery County, Md.) or with sodium fluoride (Grand Rapids, Mich.).

In the case of Montgomery County adults, the water fluoride and the urinary fluoride concentrations became virtually equal within 1 week after introduction to the fluoridated water. In school children aged 5 through 14 years (Montgomery County) and 6 through 17 years (Grand Rapids), approximately 3 to 5 years elapsed before the concentration of fluoride in the urine reached that in the drinking water. The difference in this response of adults and children during the initial period of exposure to a fluoridated drinking water suggests that the maturity of skeletal tissue influences its capacity to retain fluoride. These results give no evidence of an essential difference in the urinary elimination of fluoride ingested either in naturally fluoridated water, in water fluoridated with sodium

TABLE 2.—*Ash and fluoride concentration of skeletal tissues of individuals receiving drinking water containing 0.1 to 8.0 p.p.m. F*

		COMPOSITE DATA (DRY, FAT FREE BASIS)				
F concentration in drinking water	(ppm)	<1.0[a]	1.0[b]	2.6[c]	4.0[d]	8[e]
Age	(yrs.)	55.5	75.6	65.9	56.0	78
Residence	(yrs.)	28.1	21.5	32.9	21.3	34
Iliac crest	No.	20	4	25	4	1
	% Ash	57.4	61.2	59.7	59.9	67.5
	% F	0.041	0.138	0.267	0.413	0.577
Rib	No.	30	4	27	4	1
	% Ash	58.8	57.4	59.7	57.5	66.9
	% F	0.050	0.139	0.257	0.398	0.630
Vertebra	No.	31	5	27	4	1
	% Ash	49.0	50.0	48.3	50.7	59.5
	% F	0.054	0.160	0.286	0.411	0.540

[a] Washington, D. C., Baltimore, Md., New York City, San Antonio, Texas.
[b] Grand Rapids, Mich.
[c] Colorado Springs, Col., and Amarillo, Texas.
[d] Lubbock, Texas.
[e] Bartlett, Texas.

fluoride, or in water fluoridated with sodium fluosilicate.

When an appreciable quantity of fluoride (5.0 mg. as sodium fluoride) is ingested at one time, it is eliminated rapidly in the urine, particularly during the following first hour.

The reduction in the fluoride content of the drinking water of Bartlett, Texas, from a high of 8.0 p.p.m. to a little more than 1.0 p.p.m. provided an opportunity to obtain evidence of the elimination of fluoride from human skeletal tissue. It was ascertained in these studies on residents of Bartlett, Texas, during a period of 27 months following the reduction of fluoride in the drinking water, that the fluoride in the urine gradually decreased from as much as 8.0 p.p.m. to approximately 2.0 p.p.m. The urinary fluoride values were considerably higher than would be expected for a corresponding group with no prior exposure to high levels of fluoride. The data indicate, therefore, that fluoride previously stored in the skeleton was being mobilized. There was some evidence that mobilization was more rapid in children than in adults.

There is other substantial evidence concerning the elimination of fluoride from the body via the urine and feces as well as the body perspiration. Five young adult men have served as subjects for studies of complete balances of fluoride in which the quantitative intake of fluoride in various forms was balanced against the fluoride eliminated in the urine, feces and body sweat. It was found that there was no significant retention of fluoride in the bodies of these young men when the total daily fluoride ingested did not exceed 4.0 to 5.0 mg. daily. The data indicate that this amount of fluoride, at least, may be ingested daily without an appreciable hazard of the body storage of fluoride. In this study it was also found that the body sweat contained 0.3 to 1.8 p.p.m. of fluoride and thus became an important means for the elimination of fluoride. The body's absorption of fluoride was dependent on the solubility of the fluoride ingested. Thus the fluoride ingested as calcium fluoride, cryolite and bone meal was absorbed appreciably less than fluoride ingested in Galesburg, Illinois, water (1.8 to 1.9 p.p.m. fluoride). In the case of the ingestion of insoluble fluorides there is an increased elimination of fluoride in the feces.

It is quite apparent in the light of the above studies, as well as from other evidence, that the fluoride ingested by the use of fluoridated water is effectively eliminated from the body particularly via the urinary excretion. This fact of fluoride metabolism is an essential safeguard and preventive against the accumulation of toxic quantities of fluoride in skeletal tissue.

DEPOSITION OF FLUORIDE IN THE HUMAN BODY

The occurrence of fluoride in the animal body has been studied extensively in both laboratory and domestic animals. Similar data on man are quite limited, for obvious reasons. The presence of fluoride in human bones, cartilage, teeth, soft tissues, blood, bile and saliva will be discussed in this section.

BONES

A recent study of the deposition of fluoride in bones which have also been examined histologically furnishes very valuable information regarding the levels of fluoride tolerated in bone without any harmful effects. Roholm found approximately 0.21 to 0.89 percent fluoride in the bones of two cryolite workers whose skeletal tissues showed evidence of increased calcification and trabeculation. Osteosclerotic changes were observed roentgenographically in two other persons whose bones contained 0.97 to 1.50 percent fluoride and 0.46 to 0.50 percent fluoride, respectively.

To add substantially to the very limited previous data, a rather comprehensive survey of the fluoride content of bones of persons exposed to drinking waters containing from 0.1 to 4.0 p.p.m. fluoride was completed within the past year at the National Institute of Dental Research. In addition, a number of bones were obtained at autopsy from the body of a person who had resided for at least 34 years in Bartlett, Tex., while the drinking water contained 8.0 p.p.m. fluoride. The bones analyzed for fluoride were also examined histologically and thus afforded an evaluation of the relationship betwen fluoride concentration and microscopic change. The fluoride analysis of some 188 bones from 70 individuals is presented in table 2. To summarize these data it may be noted that:

1. The concentration of fluoride in bones increased in an essentially linear fashion with an increase of fluoride up to 4.0 p.p.m. in the drinking water.
2. For a given level of fluoride in the drinking water, the three bones studied, i.e., the iliac crest, rib and vertebra, contained similar concentration of fluoride.

CARTILAGE

In the course of the previous study, intervertebral cartilage became available for analysis. This tissue, containing about 2 percent fat, 4 percent ash and 5 percent calcium, is essentially noncalcified. It was found that the fluoride content of the dry, fat-free cartilage from persons ingesting water containing <1.0, 1.0, 2.6, 4.0 and 8.0 p.p.m. fluoride was 0.003 percent, 0.004 percent, 0.004 percent, 0.007 percent and 0.011 percent, fluoride, respectively. This very limited retention of fluoride by this tissue is in striking contrast with that retained by the bones.

TEETH

The teeth of persons exposed to drinking water containing 0.0 to 0.3 p.p.m. fluoride contain about 0.010 percent fluoride in the enamel and about 0.024 percent fluoride in the dentin. Recent work has shown that enamel fluoride is highest on the surface and decreases with depth. Where optimal levels of fluoride are present in the drinking water, namely 1.0 p.p.m. fluoride, the enamel and dentin contain 0.013 percent and 0.039 percent fluoride, respectively. Concentrations as high as 0.106 percent and 0.214 percent fluoride have been found in enamel and dentin, respectively, where the drinking water contained 7.6 p.p.m. fluoride. There appears to be no clear difference in the fluoride content of sound and carious teeth. In general, the severity of mottling is associated with an increased concentration of fluoride in the teeth, although this relation may be affected by other factors.

SOFT TISSUE

Whereas calcified tissues by their very nature become extensive depositories of fluoride, this is not the case with soft tissues which do not ordinarily calcify. Indeed it has been shown that approximately 96 percent of the fluoride retained in the animal body is found in the bones and teeth, leaving a very small quantity to be retained in the soft tissues. Satisfactory data on the fluoride analysis of soft tissues are very meager, primarily on account of the technical difficulties involved in making an accurate analysis of the extremely small quantities of fluoride present. The most to be said at this time on the subject is that progress is being made in the analytical procedure and a certain amount of the data is valuable for comparative purposes. Specimens of heart, liver, lung, kidney, spleen, and aorta obtained from persons ingesting drinking waters containing up to 4.0 p.p.m. of fluoride have been analyzed for fluoride. The persons supplying these specimens are the same as those who supplied the bone specimens for fluoride analysis and histologic study previously referred to. The preliminary fluoride data thus far have not indicated variations in fluoride content of soft tissues which could be attributed to the fluoride content of the drinking water. On the fresh basis, most soft body tissues, with a few exceptions, contain about 1.0 p.p.m. or less fluoride. The aorta, which tends to become calcified, contained 2.1 to 37.9 p.p.m. fluoride.

Blood, Bile and Saliva

Fluoride is present in blood, bile, and saliva, and probably in other extracellular fluids at a very low concentration approximately 0.1 p.p.m. fluoride. The level of fluoride in these body fluids does not change appreciably with an increase in the fluoride concentration in the drinking water. It has been shown that the fluoride content of the saliva is very little affected by the fluoride in the drinking water and is not sufficient to suggest that it is a factor in the prevention of dental caries.

In evaluating the significance of the occurrence of fluoride in body tissues, there are the following facts to be considered. It is very probable that no body tissue is free of fluoride and all skeletal and dental tissues contain fluoride. The quantity of fluoride which is tolerated by any specific body tissue is undoubtedly a major factor in the overall tolerance of fluoride, as has been previously emphasized. Answers to this latter problem have been forthcoming in the histopathologic studies of skeletal tissues and skeletal performance data correlated with the fluoride bone analysis. From all the available data it appears that skeletal tissue has an unusually high tolerance for fluoride. An increase in bone fluoride amounting to as much as 10 to 12 times the quantities normally present seems to create no health hazard. As much as 0.5 to 0.6 percent fluoride therefore may be present in bones without harm. Quantities such as these do not accumulate in human skeletal tissues as a result of continued use of fluoridated drinking water.

While the fluoride content of soft body tissues usually remains a less reliable figure, it is equally true that no significant physiologic harm to soft tissues has been correlated with exposure to relatively high levels of fluoride in drinking water. The answer seems to be that very small quantities of fluoride are present and have no deleterious effect on soft tissue.

Specific mention perhaps should be made of kidney function in these regards. Urinary excretion of fluoride, as has been noted, is an essential feature of the body's metabolism of fluoride and any prolonged curtailment of urinary fluoride excretion might become hazardous. On the other hand it has been stated with good reason that a kidney abnormality sufficient to impair normal fluoride excretion would probably prove fatal prior to any development of chronic fluorosis.

The other consideration to be mentioned concerns the kidney and its excretory function as related to a fluoride drinking water. The urinalyses included in the Bartlett-Cameron Study, the Newburgh-Kingston Study (see below) and other surveys have failed to reveal any kidney dysfunction attributable to the ingestion of fluoride water. Similarly, all the mortality statistics available indicate that exposure to a fluoride drinking water has no relation to kidney disease.

EFFECTS OF FLUORIDE ON HEALTH STATUS

In many areas throughout the world there are large populations of people who for many years have used drinking waters containing 1.0 p.p.m. or more fluoride. In the United States approximately 1,903 communities, representing about 7 million people, are now using a communal water supply which contains naturally from 1.0 up to 4.0 p.p.m. of fluoride.

Many of these persons have been studied and, in addition, there is the early classic study by Roholm on chronic fluorosis produced by a dust-borne mineral, cryolite.

In this section the information pertaining to the health status of human subjects exposed to fluoride is presented in a more or less historical relation. For obvious reasons our greatest interest lies in results pertinent to populations exposed for many years to drinking waters which contain fluoride. Much of the more significant information pertaining to human experience with fluoride waters, however, has appeared in more recent years. Several of the significant studies remain to be published in detail in the very near future.

Skeletal Fluorosis

In 1937, Dr. Kaj Roholm, of Copenhagen, Denmark, described in his monograph, "Fluorine Intoxication," the effects of fluoride poisoning as seen in a group of workers exposed for prolonged periods of time to cryolite dust. Gross abnormalities in bones and failures in skeletal performance were apparent. The workers complained of rheumatic pains, stiffness in joints and particularly in the spine. Increased bone density varying with the length of the exposure to the cryolite dust was observed by X-ray examination.

A most important part of this survey of human fluorosis was an autopsy study on two persons exposed to cryolite dust 9 and 24 years, respectively. The skeletal effects of this fluoride exposure, estimated to amount to 25.0 to 30.0 mg. daily, were characterized histologically as a diffuse osteosclerosis with pathologic formations starting in periosteum and endosteum. There was considerable formation of new bone from periosteum, and ligaments not normally calcified underwent a considerable degree of calcification.

Prolonged exposure to 10.0 p.p.m. or more fluoride in drinking water also appears responsible for a chronic skeletal fluorosis characterized by rigidity of spine, stiffness and immobility of the joints and other symptoms similar to what was observed in the cryolite workers. These defects were reported to be endemic in certain areas of South Africa and India.

Dental Fluorosis

Dental fluorosis, frequently designated as "mottled enamel," is the only known water-borne disease in dentistry. As such it is limited to definite geographic areas. Dental fluorosis is unique also as an epidemiologic disease insofar as it results only if exposure to the causative factor, i.e., a fluoride water, is essentially continuous during the period of tooth formation. The importance of the relation of water fluoridation to the production of dental fluorosis is obvious and has received careful inspection and evaluation. A most interesting facet of the dental caries-fluoride water relationship, it may be recalled, is the fact that the beneficial effect of water-borne fluoride on dental caries was first observed incidental to the early studies on the cause of mottled enamel.

Dental fluorosis has been classified in five different grades depending on its severity. Most significant is the well documented evidence concerning the quantity of fluoride in drinking water which produces dental fluorosis in these different grades of severity. Grades of mottling defined for determining the mottled enamel index of a community are "questionable," "very mild," "mild," "moderate," and "severe." It is known that at a concentration of 1.0 to 1.5 p.p.m. of fluoride in drinking water, "questionable" or "very mild" mottling occurs in a small percentage of the population. "Very mild" mottling is

Table 3.—*Endemic dental fluorosis (mottled enamel) related to content of fluoride in drinking water*

CITY	F IN PPM	NO. CHILDREN EXAMINED	COMMUNITY INDEX OF FLUOROSIS[a]	PREVALENCE (PER CENT)[b]
Colorado Springs	2.6	404	1.3	73.8
Galesburg, Ill.	1.9	273	0.7	47.6
Elmhurst, Ill.	1.8	170	0.7	40.0
Maywood, Ill.	1.2–1.5	171	0.5	33.3
Aurora, Ill.	1.2	633	0.3	15.0
Kewanee, Ill.	0.9	123	0.3	12.2
Peublo, Col.	0.6	614	0.2	6.5
Elgin, Ill.	0.5	403	0.2	4.2

[a] As described by Dean in Fluorine and Dental Health, 1942.

[b] Does not include a questionable diagnosis.

Table 4.—*Dental fluorosis in 12–14 year old children of Grand Rapids, in 1944 as compared with 1956 after 12 years of continuous exposure to fluoridated water*

YEAR	NO. OF CHILDREN EXAMINED	NORMAL	FLUOROSIS DIAGNOSIS					
			QUESTIONABLE		VERY MILD		MILD	
		No.	No.	%	No.	%	No.	%
1944	5074	4988	59	1.2	23	0.5	4	0.1
1956	755	685	44	5.8	26	3.4	0	0.0

Table 5.—*Results of dental examinations of 438 children in Newburgh and 612 children in Kingston, aged 7–14 years, after 10 years of exposure to fluoridated water in Newburgh*

	NO FLUOROSIS	ENAMEL OPACITIES DUE TO FLUOROSIS			NON-FLUORIDE OPACITIES
		QUESTIONABLE	VERY MILD	MILD	
Newburg	360	46	26	6	36
Kingston	608	4	—	—	115

recognized with difficulty as small, opaque white areas scattered irregularly or streaked over the tooth surface. Principally observed on labial and buccal surfaces, very mild mottling involves less than 25 percent of the tooth surfaces of the teeth affected. Small white areas are frequently found on the summit of the cusps. No brown stain is present.

The relation of mottled enamel to the fluoride content of drinking water is shown in tables 3, 4 and 5.

It is apparent from these data that a level of 1.0 p.p.m. fluoride in fluoridated drinking water had little effect on the prevalence of mottled enamel. This agrees with the extensive epidemiologic surveys concerning the relation of natural fluoride water to dental fluorosis. There is no esthetic significance attached to a mild, very mild or questionable form of mottled enamel. It requires a trained eye to detect these degrees of mottling. The average dental practitioner would have difficulty finding them. None of these categories of dental fluorosis is disfiguring.

Skeletal Development

In conformity with the knowledge that calcified tissues are intrinsically retentive of fluoride, a great deal of data have been obtained pertaining to skeletal development and performance. Thus, the relation between the fluoride content of drinking water (containing 0.3 to 5.6 p.p.m. fluoride) and the bone-fracture experience, height and weight of a large number of high school boys and young adult males was studied. No effect of the fluoride content of the drinking water was apparent from this survey. Fluoridated water also has had no effect on the height and weight of children. Pertinent to skeletal calcification and bone development also was a roentgenographic study of the hand and wrist of 2050 children aged 7 to 14 years exposed to 0.1 p.p.m. of fluoride vs. 3.2 p.p.m. up to 6.2 p.p.m. fluoride in their drinking water. From the roentgenograms of these children the skeletal age was assessed and a quantitative index of ossification was determined. No evidence was obtained which would indicate that there was any adverse effect on the carpal bones or on their growth and development as a consequence of the continuous use of the relatively high fluoride drinking waters. Neither was there any indication that these large quantities of water-borne fluoride caused any hypercalcification or advanced skeletal maturity in these children.

The Bartlett-Cameron Study

A comprehensive survey has been made of the health of persons exposed to 8.0 p.p.m. of fluoride present in the water of Bartlett, Tex., compared to a similar group of individuals exposed to 0.4 p.p.m. fluoride in the drinking water of Cameron, Tex. The participants had resided in these two towns for at least 15 years when first examined in 1943. A second examination was made in 1953. Included in this investigation was a full medical history, physical, dental and X-ray examination, and detailed urine and blood analyses. There was no tendency toward higher rates in these two population groups with respect to specific systemic abnormalities or laboratory findings, excepting a higher incidence of dental fluorosis in Bartlett (8.0 p.p.m. fluoride). The difference between the age-corrected death rates of the two groups was not statistically significant. Thus, no clinically significant physiologic or functional effects could be attributed to a prolonged ingestion of drinking water containing as much as 8.0 p.p.m. of fluoride. With regard to deleterious bone changes, none were produced by ingestion of this high fluoride water. There was no unusual incidence of bone fractures, arthritis, hypertrophic bone changes, or exostoses, or interference with fracture healing. There were no cases of "poker spine" and no associated functional or systemic effects.

It may be noted that the excessive fluoride in this water supply did produce roentgenographic evidence of bone changes in 10 to 15 percent of the Bartlett population group. However, these changes were slight, often difficult to recognize and frequently equivocal in degree.

THE NEWBURGH-KINGSTON STUDY

In 1945 the water supply at Newburgh, N.Y., was fluoridated to a level of 1.0 to 1.2 p.p.m. fluoride. The water of nearby Kingston remained unfluoridated as a control city. A comprehensive 10 year study of 500 Newburgh children and 405 Kingston children was reported in December, 1955. Annual medical examinations of the children and roentgenograms of the right wrist, both knees and lumbar spine were made to check the growth and development of these children over a ten year period. In addition, blood counts, urine analyses, and examinations of eyes and ears were included. A final analysis of all the data showed there were no differences of any significance (except for caries incidence) in the two groups of children, one having used a fluoridated water, the other a practically fluoride-free water.

PATHOLOGIC STUDIES IN MAN

Exposure to 2.5 p.p.m. Fluoride in Drinking Water.—One of the better known cities of the United States whose community water supplies contain in excess of 1.0 p.p.m. fluoride is Colorado Springs, Colorado. For many years approximately 2.5 p.p.m. of fluoride have been present in this city water. Results of a series of 904 necropsies performed in Colorado Springs during the years 1947–1953 have been tabulated and analyzed statistically. Since this initial study paid no special attention to the bones, a subsequent study including a detailed examination of the bones was completed between 1955 and 1957. The necropsy protocols and the diagnoses were classified according to (*a*) major cause of death, (*b*) contributing causes unrelated to the major cause, and (*c*) incidental pathology. Tabulated according to duration of residence in Colorado Springs, the data were analyzed statistically.

There were 334 individuals who had resided in Colorado Springs more than 20 years; 130 residents from 5 to 20 years; 188 residents under 5 years.

A final statistical analysis of the pathologic observations did not reveal any significant effects of a prolonged residence in the Colorado Springs environment.

Exposure to 1.0–4.0 p.p.m. Fluoride in Drinking Water.—The bone specimens for this study were obtained at necropsies performed in Grand Rapids, Michigan (1.0 p.p.m. fluoride added as sodium fluoride), Colorado Springs (2.5 p.p.m.), Amarillo, Texas (2.8 p.p.m.), and Lubbock, Tex. (4.0 p.p.m.). There was a minimum of 10 years of use of these fluoride waters by these individuals. There were 24 cases from Colorado Springs, another 13 cases were from the other localities. The ages ranged from 36 to 90 years.

Thirty-three persons from nonfluoride areas served as controls, ranging in age from 21 to 87 years. The bones examined were the body of a lumbar vertebra, a portion of the iliac crest, and the sixth rib. The lumbar intervertebral cartilage and some specimens of sternum, calvarium and femur were also studied. In all, a microscopic examination was made of approximately 100 bone specimens of persons exposed to water containing 1.0 to 4.0 p.p.m. of fluoride, and 94 bone specimens were provided by persons having less than 0.6 p.p.m. of fluoride in their drinking water.

As a result of these microscopic bone studies it became apparent that the fluoride content of the drinking water had had no effect on the histologic structure of the bones. There were microscopic changes in the bones and joints associated with aging and other conditions, but these showed no relation to the fluoride ingested in the drinking water.

In a preceding section of this discussion there is a report of the fluoride content of the majority of these same bone specimens. As noted there, the quantity of fluoride in these bones was greatly increased by the fluoride drinking water. Even with this greatly increased fluoride content, the bones showed no abnormal structures.

MORTALITY STATISTICS

A comparison of the mortality data of various populations furnishes an important index of their general health. Recently a number of carefully controlled studies have obtained mortality rates for heart disease, cancer, nephritis, diabetes, extracranial lesions and cirrhosis of the liver among communities using essen-

TABLE 6.—*Mortality rates per 100,000 population (adjusted for age, race and sex)*

DEATH	"FLUORIDE" CITIES	CONTROL CITIES
Heart disease	354.8	357.4
Cancer	135.4	139.1
Intracranial lesions	111.5	104.8
Nephritis	21.9	26.9
Cirrhosis of the liver	6.6	8.2
All causes	1,010.6	1005.0

TABLE 7.—*Ratio of recorded to expected deaths in five cities ingesting a fluoridated drinking water*

CITY AND YEAR OF FLUORIDATION	YEAR	NUMBER OF DEATHS		RATIO OF RECORDED TO EXPECTED DEATHS
		RECORDED	EXPECTED	
Grand Rapids 1945	1940	1643	1674	0.98
	1950	1803	1764	1.02
Sheboygan, Wisc. 1946	1940	394	380	1.03
	1950	383	416	0.92
Evanston, Ill. 1947	1940	611	660	0.93
	1950	672	765	0.88
Madison, Wisc. 1948	1940	546	612	0.89
	1950	652	762	0.86
Charlotte, N.C. 1949	1940	806	782	1.03
	1950	964	933	1.03

tially fluoride-free drinking water and those using waters containing fluoride naturally present or added in controlled amounts.

In one study the mortality statistics were analyzed for 32 cities whose water supplies contained more than 0.7 p.p.m. fluoride from natural sources. Each of these cities was randomly paired with a neighboring city containing less than 0.25 p.p.m. fluoride in its water supply. The total population for all cities exceeded 2 million. The death rates, adjusted for age, sex and rate, are presented in table 6; they show no significant differences related to fluoride in the drinking water.

In another study, the mortality experience in five cities was compared before and after institution of fluoridation of the water supplies to 1 p.p.m. fluoride. The cities were fluoridated during the period 1945–1949 and the number of deaths (exclusive of accidents, suicide, and homicides) in 1940 and in 1950 was recorded. These were compared with the expected deaths using the U.S. age-race-sex mortality experience as a standard. A significant change in the ratio of recorded to expected deaths for the two periods 1940 and 1950 would then indicate that some variable other than age, race, sex and declining mortality for any reason had some influence. As shown in table 7, there was no significant change in the ratio of recorded to expected deaths between 1940 and 1950.

These statistics thus indicate that no relationship existed between mortality experience and the presence of fluoride in drinking waters, whether naturally present or mechanically added. These data provide additional evidence that fluoridation of a water supply to 1.0 p.p.m. fluoride presents no health hazard.

SUMMARY

A discussion is presented on the physiologic effects of fluoride as related to water fluoridation. The data pertain essentially to clinical and epidemiologic studies and mortality statistics based on human population groups. Because of the widespread occurrence of fluoride in many community water supplies, many thousands of human subjects have been exposed to fluoride in drinking water for long periods of time prior to the advent of water fluoridation. The health status and studies on clinical material obtained from these large population groups exposed to natural fluoride waters as well as to fluoridated waters have given no evidence that a health hazard will arise owing to a level of 1.0 p.p.m. fluoride in drinking water. Highly significant dental health benefits resulting from the use of fluoridated drinking water have been conclusively established.

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DOMESTIC WATER AND DENTAL CARIES. III. Fluorine in Human Saliva

F. J. McClure

The presence of 0.095 part of fluorine per million in a pooled sample of saliva collected from three girls aged 8 living in Galesburg, Ill., and 0.120 part per million in a pooled specimen collected from three girls of the same age living in Quincy, Ill., has been reported by Cox (*1*). More recently, Wills (*2*) reported that radioactive fluorine administered intravenously to a cat appeared within one minute in the saliva of the submaxillary glands. Boissevain and Drea (*3*), however, found no fluoride in human saliva collected in an area where mottled enamel was prevalent.

The specimens of saliva for which data are given in table 1 were collected by paraffin stimulation and represent continuous secretions of approximately 100 cc. of saliva. The samples reported on in table 2 were pooled specimens collected from groups of white public school children, comprising from 40 to 200 persons per group, who were the subjects of epidemiologic studies of dental caries (*4*). The specimens of saliva in this case were collected by paraffin stimulation, but only 10 to 15 cc. was collected from each subject, part of which went into the pooled sample for analysis. All samples were preserved with thymol and were placed in the cold as soon as possible after collection.

Analysis for fluorine was made on a 100 cc. sample of saliva, which was placed in a platinum evaporating dish, made alkaline with dilute sodium hydroxide and evaporated to dryness on a water bath. Five cubic centimeters of 5 percent magnesium acetate were added and the sample dried and ashed at 500° C. The fluorine content was determined on the ash by distillation from perchloric acid and titration with standard thorium nitrate. Further details of the method are contained in an article by McClure (*5*). The percentage recoveries of 0.10 part of fluorine per million, which was added to three samples of saliva collected in Garretsville, Ohio, equaled 85, 95 and 82. If a 100 cc. sample of saliva is used for analysis the method is considered only approximate for quantities of fluorine of the order of 0.10 part per million or less. By the same procedure, three samples of cow's milk obtained from a local dairy were found to contain 0.16, 0.13 and 0.14 part of fluorine per million respectively. These results are similar to those reported by Phillips and Hart (*6*).

The data shown in table 1 indicate that the fluorine content of the saliva of normal adults (specimens obtained in Washington, D.C., and vicinity) averages about 0.10 part per million. The specimens of saliva obtained from school children of Amarillo, Texas (where there were 3.8 to 4.0 parts of fluorine per million in the drinking water) showed a fluorine content slightly above 0.10 part per million. If one considers the results of analysis of pooled specimens of saliva (table 2) collected in various cities in which the drinking water varied in fluorine content from 0.0 to 1.8 parts per million, the data cannot be said to show a consistent effect of the level of fluorine in the drinking water on the fluorine content of human saliva. The results presented in table 2, like those in table 1, indicate that the quantity of fluorine in normal saliva is equal to about 0.10 part per million.

It is to be noted in this connection that according to the data presented by Dean and coworkers (*4d*), the Illinois cities of Elmhurst, Joliet, Maywood, Aurora, Evanston and Oak Park showed pronounced differences in their dental caries experience rate. According to the results for the fluorine content of the saliva of subjects representing these respective cities (table 2), an increased amount of fluorine in the saliva is not responsible for the observed differences in the caries rates of these cities. Likewise, similar

Table 1.—*Fluorine content of saliva*

		Location	Fluorine in Drinking Water, Parts per Million	Fluorine in Saliva, Parts per Million
Samples* from adults				
F. J. Mc.	1939	Washington, D. S.	0.0†	<0.10‡ (0.04-0.09)
F. J. Mc.	1940	Washington, D. C.	0.0	0.10
C. C.	1939	Washington, D. C.	0.0	0.12
A. K.	1939	Washington, D. C.	0.0	0.12
W. T. H.	1939	Washington, D. C.	0.0	0.14
H. D.	1939	Washington, D. C.	0.0	0.12
E. E.	1939	Washington, D. C.	0.0	0.12
C. M.	1940	College Park, Md.	...	<0.10 (0.05)
A. H.	1940	Baltimore	...	<0.10 (0.07)
W. H.	1940	Washington, D. C.	0.0†	<0.10 (0.08)
Samples* from children				
No. 1	1938	Amarillo, Texas§	3.8-4.0	0.19
No. 2	1938	Amarillo, Texas	3.8-4.0	0.17
No. 3	1938	Amarillo, Texas	3.8-4.0	0.19
No. 4	1938	Amarillo, Texas	3.8-4.0	0.18
No. 5	1938	Amarillo, Texas	3.8-4.0	0.24
No. 6	1938	Amarillo, Texas	3.8-4.0	0.17
No. 7	1938	Amarillo, Texas	3.8-4.0	0.20
No. 8	1938	Amarillo, Texas	3.8-4.0	0.15
No. 9	1938	Amarillo, Texas	3.8-4.0	0.14
No. 10	1938	Amarillo, Texas	3.8-4.0	0.16
No. 11	1938	Amarillo, Texas	3.8-4.0	0.13

* The individual specimens were of 100 cc. each.

† One test made on this water showed a negative fluorine content, that is, not more than 0.10 parts per million.

‡ Average fluorine content of 10 100 cc. specimens.

§ Collected in January 1938, during a study of dental caries conducted by the Division of Infectious Diseases and Child Hygiene Section, Division of Public Health Methods.

quantities of fluorine were found in specimens of saliva collected at Galesburg and at Quincy, Ill., in 1938. (Data reported by Cox (*1*) also indicated that there were similar quantities of fluorine in the saliva of subjects from these two cities.) The caries rate for Galesburg, however, was 201 per hundred children and for Quincy 633 per hundred children.

No conclusions appear to be justified at this time with regard to the significance of the quantity of fluorine which is normally present in saliva. Any function which this normal quantity or any slight excess above normal may have as a partial preventive of dental caries remains to be demonstrated. More data are needed, also, to establish any relation between the fluorine content of the drinking water and the resulting excretion of fluorine in the saliva.

TABLE 2.—*Fluorine content of pooled specimens of children's saliva*

Location	Year	Fluorine in Drinking, Water, Parts per Million	Fluorine in Saliva, Parts per Million	No. of Specimens Pooled	Comment *
Galesburg, Ill.	1938	1.8	0.16	82	Specimens obtained from 12, 13 and 14 year old children
Quincy, Ill.	1938	0.2	0.14	63	Specimens obtained from 12 to 14 year old children
Arlington, Va., October	1939	0.0	0.10	128	Specimens obtained in three different months were from the same 12 to 14 year old children
Arlington, Va., December	1939	0.0	<0.10 (0.06)	116	
Arlington, Va., March	1940	0.0	<0.10 (0.07)	126	
Elmhurst, Ill.	1940	1.8	0.16	150	Specimens obtained from 12 to 14 year old school children
Joliet, Ill.	1940	1.3	0.10	200	Specimens obtained from 12 to 14 year old school children
Maywood, Ill.	1940	1.2	0.10	125	Specimens obtained from 12 to 14 year old school children
Aurora, Ill.	1940	1.2	0.10	75	Specimens obtained from 12 to 14 year old school children
Evanston, Ill.	1940	0.0	<0.10 (0.08)	200	Specimens obtained from 12 to 14 year old school children
Oak Park, Ill.	1940	0.0	<0.10 (0.07)	150	Specimens obtained from 12 to 14 year old school children
Bauxite, Ark.†	1940	0.0	0.15	50	Specimens obtained from 14 to 22 year old subjects with mottled enamel
Bauxite, Ark.	1940	0.0	0.10	49	Specimens obtained from 8 to 13 year old subjects not having mottled enamel
Benton, Ark.	1940	0.0	<0.10 (0.06)	41	Specimens obtained from 14 to 21 year old subjects not having mottled enamel
Garrettsville, Ohio....	1940	0.7	0.11	80	Specimens collected from the same 9 to 14 year old children on succeeding days
Garrettsville, Ohio....	1940	0.7	0.13	80	

* All of the children were white.

† The public water supply of Bauxite prior to 1928 contained about 14 parts of fluorine per million.

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FLUORIDE DOMESTIC WATERS AND SYSTEMIC EFFECTS. I. Relation to Bone-Fracture Experience, Height, and Weight of High School Boys and Young Selectees of the Armed Forces of the United States

By F. J. McClure

The involvement of skeletal tissue as an effect of toxic quantities of fluorine has been noted in a number of clinical and epidemiological studies (*1, 2*). The results of two experiments, one related to dairy cattle (*3*), the other to swine (*4*), have attributed reduced breaking strength of long bones to excessive fluorosis. On the contrary, an increase in breaking strength of long bones of dairy cattle (*5*) has been related to an enlargement of shaft walls which frequently accompanies extreme cases of fluorine toxicosis. Sometime ago it was suggested by Christiani (*6*) that fluorine might cause bones to become fragile, a suggestion which gains support from evidence that toxic skeletal effects of fluorine resemble osteosclerosis. There is the implication, therefore, that even in young individuals exposure to fluorine may be indirectly responsible for an unusual number of bone fractures. Involvement of skeletal tissues from excessive fluorine as shown in certain other results of clinical studies (*1, 2, 7, 8, 9, 10, 11*), as well as in results of certain animal experiments (*3, 4, 12, 13, 14, 15, 16*).

In order to obtain information regarding possible skeletal effects of dietary fluorine, an attempt has been made to discover a relation between fluorine ingestion via domestic drinking waters and the height, body weight, and bone-fracture experience of selected groups of 1,458 high school boys and 2,529 young adult men taking the physical examination at United States armed forces induction centers. The significance of these studies relates to two facts: (a) many drinking waters in the United States and other parts of the world contain fluorides (*17*);

and (b) it has been suggested that optimum quantities of fluoride might be added to domestic water supplies (*18, 19*) or directly to children's diets (*20*) for the partial control of dental caries. Any information which suggests a health hazard surrounding either natural or artificial sources of fluorine in human diets is of obvious interest.

PLAN OF STUDY

Procedure for obtaining physical data.—Bone-fracture data, presented in table 3, were obtained by personal interviews with selectees who reported for physical examination at induction centers located in Lubbock, Tex.; Oklahoma City, Okla.; Indianapolis, Ind.; Fort Myer, Va. (Washington, D.C., area); and Manchester, N.H. Each man was questioned as to birthplace, continuity of residence, age (as of last birthday), and bone-fracture experience. In most instances attention was called to specific body members (arm, rib, nose, collarbone, etc.). In many cases the selectee was asked to describe the nature or seriousness of his injury, in order to determine, if possible, that a fracture had actually been sustained. In general, it is believed that major breaks were accurately reported, and the data as obtained are satisfactory for comparative purposes. Height and weight (without clothing) were measured by Service personnel as a regular routine in the physical examination. Height was measured by a measuring rod attached to the weight scale.

The data shown in table 4 were copied from Army records on file at the Lubbock, Tex., induction center. The physical examination of these 935 men is assumed to be reasonably uniform. Practically all the men whose records were copied passed through the Lubbock induction center within the last 6 months of 1942 and the first 6 months of 1943.

The procedure followed for the high school boys listed in table 2 was similar to that followed at the induction centers. Boys aged 15 to 17 years (age as of last birthday) were interviewed at high schools located in Galesburg, Monmouth, Quincy, Aurora, Elgin, and Waukegan, Ill., and Washington, D.C. Only boys who gave their residences as practically continuous in their home towns were accepted for final data. Each boy was asked to state his history of bone fractures. Height was measured by a rod attached to the weight scale. Height and weight (without clothing) were taken by the writer or, under the writer's supervision, by a boy selected from each group. These groups consisted of 15 to 20 boys each. It should be noted that the weight scale with height rod was the one currently in use in the schools. The writer's height and weight were checked on the scale and height rod of each school. No appreciable variation was apparent from one school to another.

In addition to obtaining the above physical data at the induction centers and high schools, a large number of urine specimens were obtained for fluorine analysis. These analytical fluorine data are reported in a subsequent article, the second in this series.

Fluoride exposures in various areas studied.—The selection of areas of fluoride and nonfluoride exposure was made on the basis of (a) previous studies of endemic dental fluorosis, particularly by H. T. Dean and his associates (*17, 21*), and others (*22, 23*), (b) water fluoride chemistry (*23, 24, 25, 26, 27, 28*) (see also table 1), and (c) incidence of dental caries (*29, 30, 31, 32, 33*) (see also fig. 2).

Dean, Dixon, and Cohen (*21*) studied the incidence and severity of mottled enamel in the Panhandle-West Texas area in 1934. They concluded that this area "constitutes the largest mottled enamel area in the United States." A lifetime exposure to fluoride-bearing drinking water experienced by these children at approximately age 12 (*21*) is now unquestioned as the cause of their mottled enamel (*17*). Analysis of drinking waters from this locality shows the presence of unusual quantities of fluorine. (See table 1 for analytical data covering waters of this area.) The evidence of continuous fluoride exposure (mottled enamel) in children, with an average age of about 12 years, residing particularly in Texas area "A" in 1934, is good assurance that continuous residents aged 18 to 25 years in 1943 (the year of this survey) were similarly exposed to fluoride waters throughout life. Areas "A," "B," "C," and "D" (see tables 1, 3, and 4) were selected for varying levels of fluoride exposure within the West Texas-Panhandle area.

Men reporting to the Lubbock, Tex., induction center (and also to the other induction centers where this survey was made) usually arrived by county groups, and, with the exception of men from Lubbock and Amarillo, Tex., Indianapolis, Ind., and Washington, D.C., the county is the designated residence area. Many of the men in the Texas, Indiana, and New Hampshire groups came from ranches, farms, and small towns. The individual water histories of these men were not ascertained.

Residents of Texas areas "A" and "B" seem more or less certain to have been exposed to waters of high fluoride content. In general the waters of these areas come from a common aquifer, i.e., a porous sandy deposit known as the Ogallala formation (*25*). Area "C" (Gray, Donley, Hall, Motley, and Kent Counties) lies just east of the Ogallala formation. Fluorine in waters from this area averages somewhat less than 1.2 p.p.m. (table 1). Area "D" consists of Wheeler, Collingsworth, Childress, Cottle, and Stonewall Counties, east of area "C," and also includes a group of counties south and east of area "A." Water fluoride averages somewhat less than 1.0 p.p.m. F in this area (table 4). Mottled enamel is not present in endemic proportions in either area "C" or "D."

West central Oklahoma, the residence of 365 men interviewed at the Oklahoma City induction center (table 3), is desig-

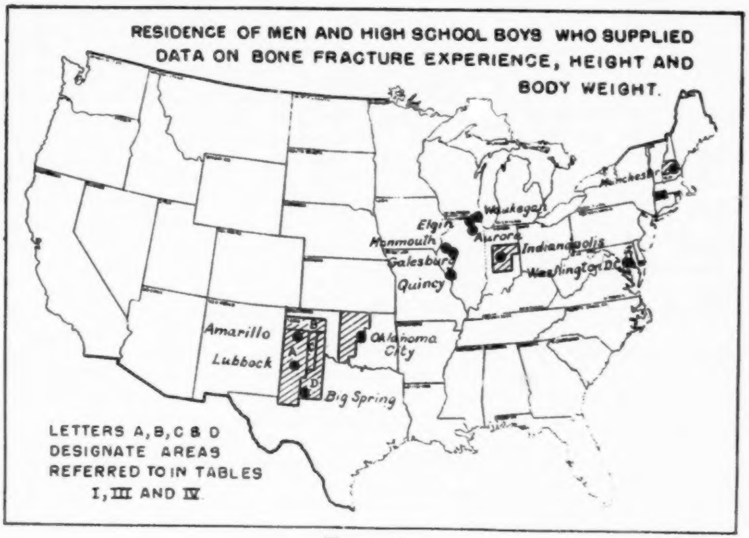

RESIDENCE OF MEN AND HIGH SCHOOL BOYS WHO SUPPLIED DATA ON BONE FRACTURE EXPERIENCE, HEIGHT AND BODY WEIGHT.
Manchester
Waukegan
Elgin
Aurora
Monmouth
Galesburg
Quincy
Indianapolis
Washington D.C.
Amarillo
Lubbock
Oklahoma City
Big Spring
A
B
C
D
LETTERS A, B, C & D DESIGNATE AREAS REFERRED TO IN TABLES I, III AND IV

FIGURE 1

nated as an area of borderline fluorosis. Mottled enamel, dental caries, and water relations of this Oklahoma area were summarized by Dean (*30*) in 1939 as follows:

> An analysis of the dental caries rates of this State (Oklahoma) as reported in Public Health Bulletin No. 226, together with observations made by one of us (H. T. D.) in connection with mottled enamel studies, indicates that the dental caries rates are appreciably lower in that part of the State in general west of the Permian outcropping. The part of Oklahoma seemingly characterized by low dental caries rates lies east of the Texas and Oklahoma Panhandles, a region where mottled enamel is generally endemic and dental caries rates low. The slope topographically is eastward. But the increased freedom from dental caries in this region may not be attributed entirely to fluoride in the water since preliminary tests have indicated concentrations of fluoride insufficient to produce considerable mottled enamel. Sporadic instances, however, of very mild mottled enamel have been observed at Lawton, Chickasha, Shawnee, and other localities in this region. This would indicate that small quantities of fluorides have been consumed by these populations.

It may be noted that Dean's observations were made in 1939; at this time (1944) it is known that fluoride insufficient to produce mottled enamel may, however, be sufficient to appreciably reduce dental caries (*29*).

The fluorine content of several communal waters from this Oklahoma area is shown in table 1.

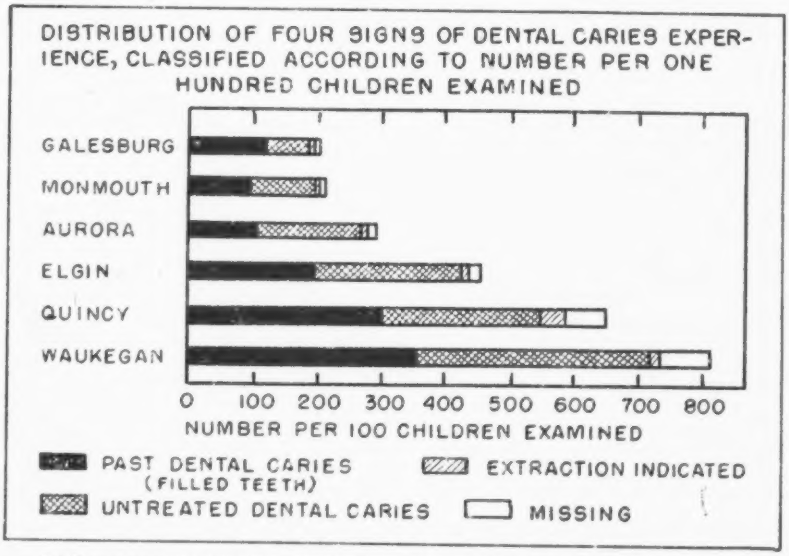
Indianapolis, Ind., and the counties included in the area designated as "rural central Indiana" (table 3) are not regarded as mottled enamel areas. The Indianapolis water supply contains currently 0.2 p.p.m. fluoride (F). Fluorine in other waters from this area is reported in table 1. The current water supply of Danville (Hendricks County), Ind., shows 1.8 p.p.m. fluorine, an unusually high figure for this area. There were, however, only 18 men from Hendricks County among the group of 232 men from rural Indiana. The fluoride exposure of these 232 Indiana men according to available evidence would appear to average about 0.5 p.p.m. water-borne fluorine.

The District of Columbia obtains its water supplies from the Potomac River. The water contains currently 0.0 p.p.m. fluorine. Endemic dental fluorosis is not present among continuous residents of the District or its suburbs.

Waters from New Hampshire (table 1) are uniformly free of fluoride. Mottled enamel is not present. New Hampshire, in common with most of the New England States, has a particularly high rate of dental caries (*33*).

Dean and his associates studied the dental health of 12- to 14-year-old children residing continuously in Galesburg, Monmouth, and Quincy, Ill., in relation to fluoride in local drinking water (*30*). This survey, which was made in 1938, should be consulted for a complete history of the communal water supplies of these three cities. Children aged 12 to 14 years residing continuously in Aurora, Elgin, and Waukegan, Ill., were subjects of a dental study by Dean and his associates in 1940 (*29*). This later report should also be consulted for a history of water supplies of these towns. Table 1 shows fluorine analysis of drinking waters used in these six Illinois towns.

Figure 2, copied from the reports by Dean, Jay, Arnold, McClure, and Elvove (*30*), and Dean, Jay, Arnold, and Elvove (*29*), shows the relative dental caries experience among 12- to 14-year-old children residing in these six Illinois towns.



DATA FOR GALESBURG, MONMOUTH AND QUINCY, TAKEN FROM REPORT BY DEAN, JAY, ARNOLD, MC CLURE AND ELVOLVE – REF. NO. 30. DATA FOR ELGIN, QUINCY AND WAUKEGAN, TAKEN FROM REPORT BY DEAN, JAY, ARNOLD AND ELVOLVE, REF. NO. 29

FIGURE 2

DISCUSSION

Athletic injuries, including incidence and types of fractures associated with various sports have been tabulated (*34*). There are also numerous statistics on fractures due to occupational, domestic, and traffic accidents (*35*); Grauhan and Schulz (*36*) classified 872 hospital-treated fracture cases according to age. They found the greatest frequency for ages 10 to 15 years. There was a surprising drop in incidence between ages 15 and 20, a sharp rise in the group aged 25 to 30, followed by a marked and continuous decline for older age groups. These workers observed that a majority of fractures were not due to major accidents or catastrophes, but to minor everyday happenings such as slipping on floors, falls from bicycles, etc., and playing games. These causes surrounded particularly the young age groups. Industrial hazards naturally played an important role among older groups. Many more fractures occurred in boys than in girls. In the 10- to 15-year age group, for example, there were 4 fractures in boys to 1 in girls (*36*).

In analyzing the data presented in tables 2, 3, and 4, and in figures 3 and 4, it is assumed that the usual activities (play, sports, etc.) which may influence the bone-fracture experience, will be similar for the various localities studied. The individual's economic status and industrial pursuits also may be presumed to affect the bone-fracture hazard. These variables, it will be noted, were not measured. In the case of New Hampshire men vs. the Oklahoma men and the Texas men, there seems some justification for regarding the outdoor activities as variable. Likewise, the communal life of the Washington, D.C., boys seems to be different from that of Illinois boys. The boys living in Washington, D.C., seem to fall into a somewhat different category than the Illinois boys, because of their residence in a large metropolitan center. In general, these boys are from families above average economic status. They were located at the Woodrow Wilson High School in one of the better residential areas of Washington. With the exception of Aurora, Ill., the Illinois

TABLE 1.—*Fluorine present in water supplies of communities in or near which the men and boys of this study have lived the major part of their lives*

Location	Date	Fluorine (p. p. m.)	Reference
Rural central Indiana			
Hendricks County:			
Danville [2]	1943	1.8	[1] F. J. M.
Brownsburg	1943	.7	27
North Salem	1943	1.0	27
Pittsboro	1943	.6	27
Jay County:			
Portland [2]	1943	.7	[1] F. J. M.
Do	1943	1.0	27
Dunkirk	1943	.9	27
Pennville	1943	1.1	27
Madison County:			
Anderson [2]	1943	.3	[1] F. J. M.
Alexandria	1943	.4	27
Chesterfield	1943	0	27
Elwood	1943	1.0	27
Frankton	1943	0	27
Pendleton	1943	0	27
Summitville	1943	1.0	27
Boone County:			
Lebanon [2]	1943	.7	[1] F. J. M.
Do	1943	1.0	27
Thorntown	1943	.6	27
Zionsville	1943	.6	27
Rush County:			
Rushville [2]	1943	.3	[1] F. J. M.
Carthage	1943	0	27
Fountain County:			
Attica	1943	.4	27
Covington	1943	.3	27
Hillsboro	1943	0	27
Veedersburg	1943	0	27
Morgan County:			
Martinsville	1943	0	27
Mooresville	1943	.3	27
Morgantown	1943	0	27
Carroll County:			
Delphi	1943	.6	27
Flora	1943	.4	27
Franklin County:			
Brookville [2]	1943	.1	[1] F. J. M.
Do	1943	0	27
Johnson County:			
Edinburg	1943	0	27
Franklin	1943	.9	27
Do [2]	1943	.4	[1] F. J. M.
Marion County:			
Indianapolis	1943	.2	[1] F. J. M.
Rural New Hampshire			
Keene [2]	1943	0.0	[1] F. J. M.
Nashua [2]	1943	0	[1] F. J. M.
Manchester	1943	0	[1] F. J. M.
Laconia [2]	1943	0	[1] F. J. M.
Newport [2]	1943	0	[1] F. J. M.
Lebanon [2]	1943	0	[1] F. J. M.
District of Columbia			
Washington	1943	0.0	[1] F. M. J
West central Oklahoma			
Cotton County:			
Walters [2]	1943	0.3	F. J. M.
Do	1932	.2	23
Welch	1932	1.5	23
Garfield County:			
Enid	1942	.4	23
Covington	1932	0.2–.3	23
Grady County:			
Minco	1934	.5	23
Rush Springs	1938	.4	23
Tuttle	1932	.3	23
Grant County:			
Wakita	1932	.3	23
Kay County:			
Newkirk	1934	0–.1	23
Love County:			
Marietta	1934	0	23
Major County:			
Fairview	1934	1.0	23
Oklahoma County:			
Oklahoma City	1934	.5	[1] F. J. M.
Do	1940	.5–1.1	23
Osage County:			
Fairfax	1932	.2	23
Foraker	1934	1.0	23
Hominy	1932	.2	23
Stephens County:			
Duncan [2]	1943	.3	[1] F. J. M.
Do	1932	1.0–2.2	23
Woods County:			
Alva	1934	0–.5	23
Woodward County:			
Woodward	1934	0	23

cities studied had but one high school, which made it possible to interview representative groups of boys. In Aurora the boys were enrolled at East Aurora High School, the larger of two city high schools. A good cross section of the community is represented among the pupils of this school. Population statistics for these Illinois towns have been discussed in reports of previous dental studies (*29, 30*).

Inspection of bone-fracture experience among the different groups of high school boys (table 2 and fig. 3) does not reveal differences of great significance. No relation can be found between fluoride exposure and incidence of fractures in these age groups. In general the data would appear to be remarkably consistent. As noted above, the data supplied by boys from Washington, D.C., may have been influenced by their economic status and facilities for athletic activities. Arm and leg fractures show a particularly uniform incidence, equalling 11 to 12 cases per 100 boys. From 50 to 60 percent of all the fractures are accounted for by arm and leg fractures. These fractures therefore occurred most frequently, and their incidence in particular indicates the ability of the important long bones to resist an outside force or unusual strain.

Other fractures, i.e., collar bone, nose, and miscellaneous fractures (ribs, fingers, toes, hands, and feet), show considerable variation, possibly because these usually constitute minor injuries and leave more varying memories. Fractures to skull, spine, and neck are included in the group of "other members fractured" (tables 2, 3, and 4). There were very few such fractures reported.

The fracture experience of different groups of Army inductees (table 2) did not bring out differences significant in themselves or related to fluoride exposure. There appears to be a slight but consistent decrease in arm, wrist, and elbow fractures, beginning with Texas men (0.5–5.2 p.p.m. F) and proceeding through fracture incidence in Oklahoma men (0.5–1.0 p.p.m. F), rural Indiana (0.0–0.5 p.p.m. F), and Indianapolis, Ind., men (0.2 p.p.m.), to Washington, D.C., and New Hampshire men (0.0 p.p.m. F) (table 3 and fig. 4). It appears wise at this time to regard the differences between New Hampshire and Washington, D.C., men and Oklahoma and Texas men, for example, as either fortuitous or possibly due to more hazardous activities connected with rural outdoor life in Texas and Oklahoma areas. Urban populations (Amarillo and Lubbock), however, showed no more fractures than Washington, D.C. Men from rural Indiana and Indianapolis possibly represent more nearly average or normal exposure to fracture-inducing activities.

The data copied from Army physical examination records on file at the Lubbock, Tex., induction center are, in general, similar to bone-fracture data obtained by the writer (table 3). It was particularly fortunate that the medical personnel at the Lubbock induction center were especially careful to determine the bone-fracture experience of men examined at that center. Inspection of the bone-fracture data (tables 3 and 4 and fig. 3) does not reveal differences among these various groups of men which may be related to fluorine exposure via drinking water.

Height and weight relations.—With the exception of Elgin boys, the average height of Illinois high school boys is 67.0 inches, varying from 66.7 inches for Aurora boys to 67.4 inches for Waukegan boys. Washington, D.C., and Elgin, Ill., boys average 68.4 and 68.0 inches respectively. Washington, D.C., boys average about 5 pounds heavier than the average weight of Illinois boys. In general these height and weight figures show no variations which are significant nor do any variations coincide with fluorine exposures. These figures compared with other height-weight data and standard for boys in these age groups are shown in table 5.

The height of Texas men averages uniformly higher than men from other areas. Men from New Hampshire average slightly more than 2 inches shorter than men from Texas and Oklahoma (tables 3 and 4). The average-weight figures for all the men are quite uniform, equaling roughly 146 to 151 pounds, excluding Oklahoma men, who average 142.4 pounds.

In general the height-weight data presented in tables 3 and 4 give no indication of a relation to fluoride exposure. A comparison of these data with other similar data and height-weight standards, is presented in table 6.

SUMMARY

A study was made of the relation of fluoride (F) in drinking water to bone-fracture experience, height, and weight of high school boys and young adult males. Among high school boys the number of fractures per 100 boys varied from 21.3 to 32.4. The numbers of frac-

tures to arms and legs per 100 boys varied from 14.0 to 18.5. These later fractures account for about 60 percent of all fractures. There was no relation of fracture experience to fluoride exposure. The average height and body weight of all the boys compared favorably with other height-weight data and accepted standards. The height-weight data were not related to fluoride exposures.

The bone-fracture experience of young adult males who were taking the physical examination at armed forces induction centers varied from about 25.0 to 30.0 fractures per 100 men averaging about 18 to 25 years of age. An experience of more than 1 fracture varied from 1.7 to 4.8 men per 100 men. While these data on bone-fracture experience for both men and boys of these ages do not permit final conclusions, they do suggest strongly that no serious impairment in skeletal performance, as might be manifest in number of broken bones, seems related to exposure to fluoride domestic waters of the concentrations studied in this survey.

Texas men exposed to highest water-fluorine concentrations and Oklahoma men averaged 69.6 and 69.4 inches in height (weight 149.0 and 142.4 pounds), respectively. Men from rural Indiana and Indianapolis averaged 68.1 and 68.3 inches in height, 146.8 and 146.2 pounds in weight, respectively. Washington, D.C., men averaged 69.3 inches and weighed 151.2 pounds on the average. New Hampshire men were 67.3 inches tall and weighed 149.6 pounds on the average. These height-weight figures showed no relation to fluoride exposure.

ACKNOWLEDGMENTS

The writer is especially grateful for the assistance given by the numerous educational authorities in each of the six Illinois cities and in the District of Columbia. Their interest and cooperation were extremely helpful. Acknowledgment is made also to the medical officers and other personnel of armed forces induction centers, where facilities were made available for this study.

PERMISSION FOR PUBLICATION

Permission for publication of the data obtained at armed forces induction centers has been granted by the Office of the Surgeon General, War Department, Washington, D.C.

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TABLE 1.—*Fluorine present in water supplies of communities in or near which the men and boys of this study have lived the major part of their lives*—Continued

Location	Date	Fluorine (p. p. m.)	Reference
Illinois			
Galesburg	1933–34	1.8	28
Do	1938	1.9	30
Do [3]	1943	1.9	[4] C. A. K.
Do [5]	1943	1.8	[4] C. A. K.
Do [3]	1943	1.8	[4] C. A. K.
Monmouth	1933–34	1.7	28
Do	1938	1.6	30
Do [3]	1943	1.7	[4] C. A. K.
Aurora	1939–40	1.2	29
Do [3]	1943	1.0	[4] C. A. K.
Do [3]	1943	.9	[1] F. J. M.
Elgin	1939–40	.5	29
Do [3]	1943	.7	[1] F. J. M.
Quincy [2]	1938	.2	30
Do [3]	1943	.1	[4] C. A. K.
Waukegan	1938	0	29
Do [3]	1943	.1	[4] C. A. K.
Texas area "A"			
Lubbock	1933–34	4.4	28
Do	1934	4.2	28
Do [3]	1943	3.8	[1] F. J. M.
Lubbock Army Air Field	1943	5.1	[1] F. J. M.
Lubbock Glider Field	1943	2.0	[1] F. J. M.
Amarillo	1933–34	3.9	28
Do	1934	5.6	28
Do	1943	5.1	[6] J. W.
Do	1943	4.3	[6] J. W.
Hereford	1941	3.2	[7] E. E.
Spur	1943	3.8	[7] E. F.
Slaton	1935	5.2	[7] E. E.
Post	1935	6.0	[7] E. E.
Levelland [2]	1943	3.4	[1] F. J. M.
Levelland	1943	3.5	26
Muleshoe	1938	1.5	26
Crosbyton	1939	2.8	26
Ralls	1938	2.4	26
Lamesa	1938	5.2	26
Do [3]	1943	3.9	[1] F. J. M.
Floydada	1943	2.5	26
Texas area "A"—Continued			
Lockney	1937	2.5	26
Seminole [2]	1943	2.3	[1] F. J. M.
Plainview	1933–34	2.9	[7] E. E.
Hale Center	1929	3.4	26
Tahoka	1938	4.0	26
Midland	1938–39	2.5–3.6	26
Texas area "B"			
Canadian	1936–38	0.8–1.2	26
Dalhart	1939	1.2	26
Do [3]	1943	.6	[1] F. J. M.
Texline	1936–39	1.0	26
Dumas	1939	1.8	26
Spearman	1937	1.8–2.3	26
Booker	1938	.6	26
Texas area "C"			
Memphis	----------	1.0–1.3	26
Lefors	1939	.6–.8	26
McLean	1938	.8	26
Pampa	1938	.8	26
Do [3]	1943	.8	[1] F. J. M.
Memphis	1936	.3	26
Estelline	1936	.6	26
Clarendon [2]	1943	.2	[1] F. J. M.
Matador	1939	1.0	26
Texas area "D"			
Big Spring	1933–34	0.7	28
Do	1934	1.1	28
Do [3]	1943	8	[1] F. J. M.
Wheeler	1938	.4	26
Do [3]	1943	.3	[1] F. J. M.
Wellington	1936	.2	23
Do [3]	1943	.4	[1] F. J. M.
Paducah	1938	.4	26

[1] F. J. McClure.
[2] Water sample supplied by superintendent, City Water Co.
[3] Water from high school tap.
[4] C. A. Kinser, associate chemist, National Institute of Health.
[5] Sample supplied by I. A. Neifert, Knox College.
[6] Jack Wyatt, city chemist, Amarillo, Tex., private communication.
[7] Elias Elvove, senior chemist, National Institute of Health, private communication.

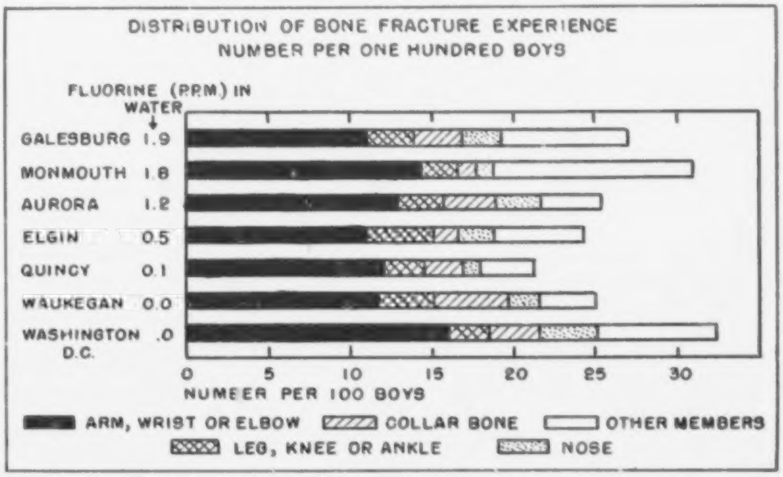

FIGURE 3

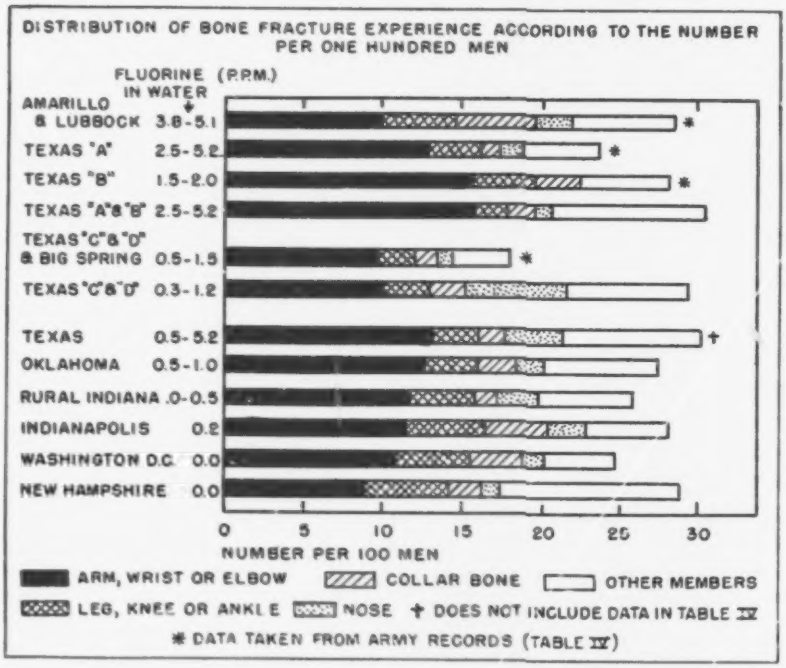

FIGURE 4

TABLE 2.—*Bone-fracture experience, height, and weight of 1,458 high school boys aged 15 to 17 years, residing in six Illinois towns and Washington, D.C., where community water supplies varied in fluorine content from 0.0 to 1.9 p.p.m.*

Location	Galesburg	Monmouth	Galesburg and Monmouth	Aurora	Elgin	Quincy	Waukegan	Quincy and Waukegan	Washington, D.C.[1]
Fluorine in water (p.p.m.)	1.9	1.7	1.7–1.9	1.2	0.5	0.1	0.0	0.0–0.1	0.0
Number of boys	207	90	297	348	218	206	203	409	286
Boys aged 15 years (percent)	37.05	41.11	38.33	45.56	30.27	22.33	46.79	34.47	30.77
Boys aged 16 years (percent)	36.04	35.55	35.89	33.46	34.86	39.32	38.92	39.12	39.51
Boys aged 17 years (percent)	26.91	23.34	25.78	20.98	34.86	38.35	14.29	26.41	29.72
Continuity of residence (years)	15.8	15.1	15.5	15.6	15.7	15.5	15.2	15.4	15.1
Average age (years)	16.1	15.8	16.0	15.9	16.1	16.2	15.7	15.9	16.0
Average height (inches)	67.6	66.5	67.2	66.7	68.0	67.2	67.4	67.3	68.4
Average weight (pounds)	138.2	129.9	135.6	136.5	136.1	134.2	135.8	135.0	140.7
BONE-FRACTURE EXPERIENCE (NUMBER PER 100 BOYS)									
Arm, wrist, or elbow	11.1	14.4	12.1	12.9	11.0	12.1	11.8	12.0	16.1
Leg, knee, or ankle	2.9	2.2	2.7	2.8	4.1	2.4	3.4	2.9	2.4
All fractures of arm and leg	14.0	16.6	14.8	15.7	15.1	14.5	15.2	14.9	18.5
Collar bone	2.9	1.1	2.4	3.2	1.4	2.4	4.4	3.4	3.1
Nose	2.4	1.1	2.0	2.8	2.3	1.0	2.0	1.5	3.5
Other members fractured	7.7	12.2	9.1	3.6	5.5	3.4	3.4	3.4	7.3
Total of all fractures	27.0	31.0	28.3	25.3	24.3	21.3	25.0	23.2	32.4
PERCENT OF BOYS HAVING BONE-FRACTURE EXPERIENCE									
Boys experiencing 1 or more fractures	23.7	22.2	23.2	19.8	21.6	18.0	19.7	18.8	23.1
Boys experiencing more than 1 fracture	2.9	4.4	3.4	5.2	2.3	3.4	4.4	3.9	4.5

[1] There were 67 boys in this group whose continuous residence was in Washington, D. C., and in one of the following cities: New York, Chicago, Baltimore, Brooklyn, Boston, Detroit, Philadelphia, and Atlantic City. There is reasonable assurance that the water supplies of these cities are practically fluoride-free.

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TABLE 3.—*Bone-fracture experience, height, and weight of 1,594 men reporting for physical examination at armed forces induction centers located in selected fluoride and nonfluoride areas of the United States*

Area classification	Fluoride			Borderline fluoride	Nonfluoride			
Fluorine in drinking water (p. p. m.)	2.0–5.0	0.3–1.0	0.3–5.0	0.5–1.0	0.0–0.5	0.2	0.0	0.0
Location	Texas area "A" and "B"	Texas area "C" and "D"	All Texas areas	West-central Oklahoma	Rural-central Indiana	Indianapolis Indiana	Washington, D. C.	Central New Hampshire
Number of men interviewed	190	138	[1] 328	365	232	210	213	246
Continuity of residence (years)	17.0	15.8	16.5	18.1	19.6	19.3	21.3	22.0
Average number of years in school	10.7	11.5	11.0	10.6	11.2	11.7	11.1	10.7
Average age (years)	19.9	18.7	19.0	18.8	20.1	19.4	21.8	22.5
Average height (inches)	69.6	69.5	69.6	69.4	68.1	68.3	69.3	67.3
Average weight (pounds)	149.7	147.9	149.0	142.4	146.8	146.2	151.2	149.6
BONE-FRACTURE EXPERIENCE (NUMBER PER 100 MEN)								
Arm, wrist, or elbow	15.8	9.8	13.3	12.6	11.6	11.4	10.8	8.9
Leg, knee, or ankle	2.1	3.6	2.7	3.3	4.3	5.2	4.7	5.3
All fractures of arm and leg	17.9	12.9	16.0	15.9	15.9	16.6	15.5	14.2
Collar bone	1.6	2.2	1.8	2.5	1.3	3.8	3.3	2.0
Nose	1.1	6.5	3.4	1.9	2.6	2.4	1.4	1.2
Other members fractured	9.9	7.9	9.1	7.2	6.0	5.2	4.7	11.4
Total of all fractures	30.5	29.5	30.3	27.5	25.8	28.0	24.9	28.9
PERCENT OF MEN HAVING BONE-FRACTURE EXPERIENCE								
Men experiencing 1 or more fractures	26.3	24.5	25.5	23.3	24.1	22.5	22.1	25.6
Men experiencing more than 1 fracture	3.2	4.3	3.6	3.8	1.7	4.8	2.8	3.3

[1] 187 of these men from Texas areas were students at the Texas Technological College, Lubbock, Tex.

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Table 4.—*Bone-fracture experience, height, and weight of 935 men reporting for physical examination at armed forces induction center, Lubbock, Tex. Data copied from Army records*

Mottled enamel index [1]	Very marked to medium			Slight	Borderline to negative				
Fluorine in drinking water, estimated (p. p. m.)	2.0-5.1			1.2-2.0	0.3-1.0				
Location [2]	Amarillo and Lubbock	Area "A"	Area "A" Amarillo and Lubbock	Area "B"	Area "C"	Area "D"	Big Spring	Areas "C" and "D" and Big Spring	All Texas areas
Number of men	178	337	515	71	98	208	43	349	935
Average years in school [3]	10.7	10.4	10.5	10.8	10.1	10.0	9.7	10.1	10.3
Average age (years)	20.4	20.7	20.6	21.5	21.3	21.2	21.4	21.3	20.9
Average height (inches)	69.1	69.3	69.2	69.0	69.3	69.4	68.9	69.3	69.2
Average weight (pounds)	149.0	146.2	147.1	149.6	148.4	149.8	147.4	149.1	148.1
BONE-FRACTURE EXPERIENCE (NUMBER PER 100 MEN)									
Arm, wrist, or elbow	10.1	12.8	11.8	15.5	11.2	8.7	11.6	9.7	11.3
Leg, knee, or ankle	4.5	3.3	3.7	4.2	3.1	2.4	0.0	2.3	3.2
All fractures of arm and leg	14.6	16.1	15.5	19.7	14.3	11.1	11.6	12.0	14.5
Collar bone	5.1	1.2	2.5	2.8	2.0	1.4	0.0	1.4	2.1
Nose	2.2	1.5	1.8	0.0	2.0	0.0	2.2	0.9	1.3
Other members fractured	6.7	4.8	5.5	5.6	3.1	3.8	4.3	3.7	4.8
Total of all fractures	28.6	23.6	23.3	28.1	21.4	16.3	17.1	18.0	22.7
PERCENT OF MEN HAVING BONE-FRACTURE EXPERIENCE									
Men experiencing 1 or more fractures	21.3	19.9	20.4	22.9	20.4	13.9	17.1	16.3	19.1
Men experiencing more than 1 fracture	4.5	2.7	3.3	2.6	1.0	2.9	0.0	2.0	2.8

[1] See Mottled enamel in Texas, by H. Trendley Dean, R. M. Dixon, and Chester Cohen. Pub. Health Rep., 50: 424-442 (March 1935).

[2] Only men were selected whose place of birth and current residence were the same.

[3] Records were copied for men who had completed a minimum of 6 years of school.

Table 5.—*Comparison of average height and weight of high school boys (table 2) with other height and weight data for boys aged 16 years*

	Height (inches)	Weight (pounds)	Reference
BOYS AGED 16 YEARS			
Baldwin-Wood tables	62-72	107-155	38
College students	67.48	133.70	37
Engelbach	61.8-66.2	96-122	39
Boys in military training camps	66.92	128.09	40
Medico-actuarial tables	(67)	(132)	41
Do	(68)	(136)	41
Do	(69)	(140)	41
BOYS APPROXIMATE AVERAGE AGE 16 YEARS			
Galesburg and Monmouth boys	67.2	135.6	Table 2
Aurora boys	66.7	136.5	Table 2
Elgin boys	68.0	136.1	Table 2
Quincy boys	67.2	134.2	Table 2
Waukegan boys	67.4	135.8	Table 2
Washington, D. C., boys	68.4	140.7	Table 2

TABLE 6.—*Comparison of height and weight of young adult male populations*

Age	19		20		21		22		Reference
	Height, inches	Weight, pounds	Height, inches	Weight, pounds	Height, inches	Weight, pounds	Height, inches	Weight, pounds	
College students	68.82	142.5	68.79	144.1	68.77	144.9	....	....	(37)
Army recruits 1906–15	66.97	136.3	67.02	138.1	67.33	141.2	67.34	....	37, 42
Engelbach	67.5	131.3	68.0	135.0	....	....	....	....	39
Men in citizen's training camps	68.07	137.7	68.10	139.9	68.16	140.9	....	....	40
Medico-actuarial tables	(67)	(138)	(67)	(140)	(67)	(142)	(67)	(143)	41
Do	(68)	(142)	(68)	(144)	(68)	(145)	(68)	(146)	41
Do	(69)	(146)	(69)	(148)	(69)	(149)	(69)	(150)	41
Texas areas "A" and "B"	....	....	69.6	149.7	....	....	....	....	Table 3
Texas areas "C" and "D"	69.5	147.9	....	....	....	....	....	....	Table 3
Texas area "A", Amarillo, Lubbock	....	....	....	....	69.2	147.1	....	....	Table 4
Texas areas "C" and "D" Big Spring	....	....	....	....	69.3	149.1	....	....	Table 4
All Texas areas	69.6	149.0	....	....	....	....	....	....	Table 3
Do	....	....	....	....	69.2	148.1	....	....	Table 4
West-central Oklahoma	69.4	142.4	....	....	....	....	....	....	Table 3
Rural-central Indiana	....	....	68.1	146.8	....	....	....	....	Table 3
Indianapolis, Ind	68.3	146.2	....	....	....	....	....	....	Table 3
Washington, D. C	....	....	....	....	....	....	69.3	151.2	Table 3
Central New Hampshire	....	....	....	....	....	....	67.3	149.6	Table 3
556 freshmen—Texas A. & M. College [1]	69.2	145.3	....	....	....	....	....	....	(F. J. Mc.)
770 freshmen—Maryland University [1]	68.8	146.2	....	....	....	....	....	....	(F. J. Mc.)

[1] Boys aged 17, 18, and 19 years. Data copied from records of physical examination given at time of entry (1940–43).

FLUORIDE DOMESTIC WATERS AND SYSTEMIC EFFECTS. II. Fluorine Content of Urine in Relation to Fluorine in Drinking Water

By F. J. McClure and C. A. Kinser

The preceding article (*1*), by one of us (F. J. McC.), reports an epidemiological study of the bone-fracture experience, height, and body weight of high school boys and young men in relation to their exposure to fluorine in drinking water. The fluorine content of urine specimens obtained from a large number of these same men and boys is reported in this article. The urine analyses also are studied in relation to the fluorine content of the local water supply.

Concentrations of fluorine permissible in domestic water and in food are now established at 1.00 p.p.m. in drinking water according to Public Health Service Drinking Water Standards (*2*) and 7.0 p.p.m. in marketable sprayed apples and pears as established by Federal Government restriction. When the concentration of fluorine in a domestic water exceeds approximately 1.5 p.p.m. (associated with an index of dental fluorosis of about 0.6) endemic dental fluorosis as stated by Dean (*3*) "begins to constitute a public health problem warranting increasing consideration." Fluoride ingested in food alone has been found "insufficient to produce even the faintest signs of dental fluorosis in as little as one percent" of a group of over 2,000 white public school children (*4*). Fruits and vegetables carrying fluorine spray residues, while not studied specifically in these regards, have given no evidence of producing dental fluorosis (mottled enamel).

An important effect of fluorine in drinking water is a marked reduction of dental caries attack in children aged 12 to 14 (*4*, *5*). It is quite possible that this effect also applies to older age groups (*6*). These effects are associated with as little as 1.00 p.p.m. fluorine in a domestic water. Absence of endemic dental fluorosis (mottled enamel) in connection with the domestic use of waters of this fluoride concentration, i.e., 1.00 p.p.m. fluorine (*4*), has made it possible to consider seriously the direct fluorination of communal water supplies in this concentration, for the partial control of dental caries (*7*). Other suggested health hazards which may surround this concentration, and also higher concentrations of fluorine in drinking water, are being investigated in studies such as those reported in this article and in the preceding article (*1*).

The public health hazard possibly connected with the domestic use of fluoride-bearing waters and which seems to deserve most serious study at this time relates to cumulative toxic bone fluorosis (*8*). This form of fluorine toxicity is attributed to the abnormal accumulation of fluorine in skeletal tissues (*8*). In connection with excessive exposures to fluorine from food or drinking water, it appears that the successful adjustment of the body to this fluorine may be largely dependent on the elimination of fluorine via the urinary excretion. For this reason the fluorine content of urine studied in relation to the fluorine exposure may be of special value as a measure of a suspected health hazard related to cumulative bone fluorosis. Urinary fluorine data may also serve as an index of the approximate fluorine exposure from food and drinking water.

Fluorine content of urine of human subjects has been studied most extensively thus far by Machle (*9*). Machle, Scott, and Treon (*10*), Machle, Scott, and Largent (*11*), and Machle and Largent (*12*). Brun, Buchwald, and Roholm (*13*) studied the excretion of fluorine in urine of cryolite factory workers. (Cryolite is the mineral Na_3AlF_6.) Shortt, McRoberts, Barnard, and Nayar (*14*) found fluorine in appreciable quantities in urine of certain natives of India exposed to fluorine in their water supplies.

The results of studies by Machle and his coworkers may be summarized as follows: According to Machle (*9*) fluorine content of urine ranges from 0.5 to 2.89 p.p.m. and averages about 1.00 p.p.m. The specimens analyzed were provided by 10 women and 19 children who were hospital patients, and by 101 subjects chosen at random. Based on a rather limited number of specimens, Machle, Scott, and Treon (*10*) report an appreciable elevation of fluorine in urine where there is an exposure to fluorine in the drinking water. In an experimental study (*11*), a normal man was found to remain in a normal or noncumulative fluorine balance when ingesting 0.4 mg. to 0.6 mg. of fluorine daily. "Measurable storage of fluorine did not occur. Over 80 percent of the fluorine was absorbed and excreted in the urine" (*11*). An equilibrium between fluorine ingestion and output was further demonstrated by these workers "under normal levels of fluoride intake," i.e., at about 0.5 mg. fluorine ingested daily (*11*). Increasing the daily fluorine intake by addition of 6.0 mg. of fluorine to the ingesta caused an increase in the urinary excretion of fluorine. However, this increased urinary excretion did not bring about the elimination of all the absorbed fluorine (ingesta fluorine minus feces fluorine). A body storage of fluorine occurred, therefore, estimated as follows in milligrams of fluorine retained daily: From sodium fluoride in drinking water, 3.87 mg.; from bone meal 1.74 mg.; and from cryolite 2.43 mg. As noted above, in these comparative tests an equal quantity (6.0 mg. daily) of fluorine was provided by each fluorine compound. The observed differences in daily retention were due mainly to differences in quantities absorbed rather than to variability in the percent of absorbed fluorine excreted in the urine.

Fluorine content of urine, according to Brun, Buchwald, and Roholm (*13*), will normally average 0.80 p.p.m., varying from 0.3 to 1.6 p.p.m. Average daily excretion equals 0.92 mg., varying from 0.18 to 1.85 mg. daily. These results are based on 24-hour urine collections from 30 hospital patients aged 2 months to 78 years. Twenty-four workers exposed to cryolite-factory dust had an average of 16 p.p.m. of fluorine in urine specimens excreted between 8 a.m. and 2:30 p.m. at the factory. These analyses varied from 2.4 to 43.4 p.p.m. fluorine and seemed proportional to exposure to cryolite dust. In this connection it is interesting to note that men who had remained away from these factories as long as 5 to 7 years, after working in the factories 24 to 28 years, continued to show as much as 3.7 to 3.9 p.p.m. fluorine in urine specimens. In these men fluorine was undoubtedly mobilized from skeletal fluorine acquired during previous fluorine exposure from cryolite dust.

PLAN OF STUDY

With the assistance of induction center personnel, urine specimens were obtained from young adult men reporting for physical examination at Army induction centers, located at Fort Myer, Va. (Washington, D. C., area), Manchester, N.H., Indianapolis, Ind., Chicago, Ill., Oklahoma City, Okla., and Lubbock, Tex. Specimens were obtained from high school boys aged 15 to 17, residing in Washington, D.C., Little Rock, Ark., Oklahoma City, Okla., Lubbock, Tex., Amarillo, Tex., and in the following Illinois cities: Waukegan, Quincy, Elgin, Aurora, Monmouth, and Galesburg. As a rule, specimens were obtained between 9 a.m. and noon. Equal volumes, usually 20 cc., of each specimen were pooled (15 to 20 or less specimens per pooled sample), according to age or duration of fluorine exposure. These samples were preserved with toluene and shipped to the National Institute of Health, U.S. Public Health Service, Bethesda, Md., for fluorine analysis.

The fluorine exposure via drinking water of the majority of these men and boys has been discussed somewhat in detail in the previous report on the bone-fracture experience, height, and body weight of these men and boys (*1*). This report should be consulted for details of fluorine exposures. In connection with the study of high school boys, a sample of the local water supply was obtained at the time of the survey. The fluorine content of these waters appears in table 2. Current water samples from a number of communities in which the inductees resided also were analyzed for fluorine, and these results appear in table 1 along with the analytical figures for fluorine in urines.

TABLE 1.—*Fluorine content of urine. Pooled specimens from men taking physical examination at armed forces induction centers located in fluoride and nonfluoride areas*

Location	Date (1943)	Fluorine in water (p. p. m.)	Fluorine in urine (p. p. m.)	Number of specimens pooled	Comment
NONFLUORIDE AREAS					
Washington, D. C.	May 21	0.0	0.3	22	Men aged 17 to 28 reporting at the Ft. Myer, Va., induction center. Lifetime residence in Washington, D. C., or suburbs.
	May 22	0	.4	22	
	May 24	0	.3	28	
	May 25	0	.5	27	
	May 26	0	.4	27	
	May 27	0	.4	27	
	May 28	0	.6	21	
	May 29	0	.4	28	
Indianapolis, Ind.	June 12	.2	.5	24	Men aged 17 to 27 reporting at Indianapolis, Ind., induction center. Lifetime residence in Indianapolis.
	June 12	.2	.4	18	
	June 14	.2	.5	19	
	June 16	.2	.3	22	
	June 16	.2	.3	20	
	June 19	.2	.3	18	
Hendricks County, Ind.	June 15	[1] 0.6–1.8	1.0	18	Men aged 18 to 25 reporting at Indianapolis induction center. Lifetime residence in respective county or town.
Jay County, Ind.	June 15	[2] 0.7–1.1	.8	21	
Anderson, Ind.	June 16	.3	.3	26	
Lebanon, Ind.	June 17	.7	.9	18	
Whiting, Ind.	June 18	.1	.4	26	
Chicago, Ill.	Nov. 13	0	.3	19	Men aged 18 to 31 reporting at Chicago induction center. Lifetime residence in Chicago.
	Nov. 13	0	.3	17	
	Nov. 16	0	.2	24	
Lebanon, N. H.	Sept. 1	0	.3	25	Men aged 17 to 30 reporting to New Hampshire induction center. Lifetime residence in respective communities.
Keene, N. H.	Sept. 1	0	.2	25	
Nashua, N. H.	Sept. 2	0	.2	24	
Lancaster, N. H.	Sept. 2	----------	.4	25	
Manchester, N. H.	Sept. 3	0	.4	25	
Laconia, N. H.	Sept. 3	0	.2	25	
Newport, N. H.	Sept. 3	0	.2	26	

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[1] Fluorine analyses of waters of Hendricks County towns are reported by Jeup (*28*) as follows: Brownsburg 0.7 p. p. m., North Salem 1.0 p. p. m., Pittsboro 0.6 p. p. m. Danville water contains (November 1943) 1.8 p. p. m. fluorine (F. J. Mc.).

[2] Jay County towns.—Fluorine analyses are reported by Jeup (*28*) as follows: Portland 1.0 p. p. m., Dunkirk 0.9 p. p. m., Pennville 1.1 p. p. m. Portland water contains (November 1943) 0.7 p. p. m. fluorine (F. J. Mc.).

Table 1.—*Fluorine content of urine. Pooled specimens from men taking physical examination at armed forces induction centers located in fluoride and nonfluoride areas*—Continued

Location	Date (1943)	Fluorine in water (p. p. m.)	Fluorine in urine (p. p. m.)	Number of specimens pooled	Comment
		FLUORIDE AREAS			
Amarillo, Tex.	July 6	[3] 4.3-5.1	3.9	27	Men aged 18 to 34. 1 year in Amarillo.
	July 17	4.3-5.1	4.0	15	Men aged 18 to 35. 1 year in Amarillo.
Lubbock, Tex.	July 7	3.8	4.2	21	Men aged 16 to 2[illegible] month to 1 year in Lubbock (Texas Technical College students); man aged 19, lifetime in Lubbock.
	July 8	3.8	4.2	1	
Lubbock Army Air Field	July 15	5.1	4.8	12	Men aged 17 to 34. 12 to 19 months at Air Field.
	July 15	5.1	3.8	7	Men aged 21 to 46. 8 to 11 months at Air Field.
	July 15	5.1	3.7	6	Men aged 22 to 37. 2 to 3 months at Air Field.
	July 15	5.1	3.6	25	Men aged 17 to 46. 2 to 12 months at Air Field.
Lamesa, Tex.	July 12	[4] 3.9	4.5	11	Men aged 18 to 35 years reporting at Lubbock, Tex., induction center. 1-year residence in respective county or town.
Levelland, Tex.	July 17	[4] 3.5	4.8	6	
Gaines County, Tex.	July 9	....	3.7	7	
Dallam County, Tex.	July 13	....	4.0	12	
Hutchinson County, Tex.	July 8	....	4.0	12	
Pampa, Tex.	July 9	[4] .8	3.7	15	
Clarendon, Tex.	July 7	[4] .2	3.3	19	
Wheeler, Tex.	July 8	[4] .3	2.3	18	
Wellington, Tex.	July 16	[4] .4	3.0	8	
Lubbock Glider Field	July 17	2.0	2.0	8	Men aged 20 to 35. 1 to 4 months at Glider Field.
	July 17	2.0	1.9	8	Men aged 22 to 36. 1 to 4 months at Glider Field.
Joliet, Ill.	Nov. 13	1.3	1.0	21	Men aged 22 to 37 (entire life in Joliet).[5]

[3] Amarillo city water (August and September 1943) analyses by J. C. Wyatt, City Chemist, Amarillo, Tex.

[4] It should be noted particularly that these men, although exposed to these waters at home, as a general rule arrived in Lubbock, Tex., the afternoon or evening prior to the morning on which the urine specimens were obtained. During their stay in Lubbock they were exposed to Lubbock drinking water, which contained 3.8 p. p. m. fluorine. The rapid response of urinary fluorine to fluorine ingested in drinking water is shown by the data in table 3.

[5] These men were taking the physical examination at the Chicago, Ill., induction center, and were in Chicago several hours at least prior to obtaining these urine specimens. Chicago drinking water is fluoride-free.

DETERMINATION OF FLUORINE

The volume of urine for fluorine determination was 100 ml., with the exception that 50 or 25 ml. were taken if the fluorine content was expected to be considerably above normal. The rule followed was to have preferably 50 to 100 micrograms of total fluorine in the analytical sample. Although 100-ml. quantities of urines from nonfluoride areas contained only 25 to 50 micrograms, these quantities were determined with the desired accuracy. The urine sample was made slightly alkaline with sodium hydroxide, 2.5 ml. of 5 percent magnesium acetate added, the sample evaporated to dryness in a platinum dish, and then ashed at 500° C. Moistening the ash with water, drying, and ashing again facilitates the ashing process. This ashed sample was transferred to a 50-ml. Claissen flask containing a glass bead and a pinch of silica, using water and 5 or 6 ml. of 60 percent perchloric acid. Total volume of washing water and perchloric acid amounted to about 15 ml. The sample was thoroughly disintegrated and a solution of silver perchlorate (1.0 gm. per ml.) added slowly to precipitate chlorides. This addition of silver perchlorate was roughly quantitative, i.e., until all the chloride in the sample appeared to be precipitated. A slight excess of silver perchlorate, however, has not been found to interfere with the determination (*15*). The fluorine was steam-distilled from this mixture, 150 ml. being distilled, the distillate kept alkaline to phenophthalein with 5 ml. of 0.10 normal sodium hydroxide. This volume was distilled over in about one-half hour. Some bumping may occur during the early stages of this distillation but can be kept under control so as not to interfere with the determination. The 150 ml. of distillate was evaporated in a platinum dish almost to dryness, made slightly acid, and transferred to a 10-ml. volumetric flask. A one-fifth or one-tenth aliquot of this solution was then titrated with standard thorium nitrate. This is essentially the method of Willard and Winter (*16*) modified by Armstrong (*17*). A study of this method by McClure (*15*) has been reported. The accuracy of this procedure with respect to urine samples is approximately ±0.2 p.p.m. The recovery of known quantities of fluorine added to 100-ml volumes of urine was as shown in Table 1a. Fluorine was determined in water supplies by this method, using 100 ml. as the analytical sample.

DISCUSSION

Urinary fluorine in specimens from over 1,900 men and boys representing a variety of conditions ranging from fluoride-free to high fluoride domestic-water areas, has been studied. A remarkable relationship was observed between urinary fluorine and fluorine content of domestic water. Beginning with exposures approximating 0.5 p.p.m. fluorine in the local domestic water, urine specimens show a detectable increase in fluorine. Fluorine in urine continues proportional to water fluorine up to about 5.0 p.p.m. fluorine in the local water supply (fig. 1).

The sensitivity of urinary fluorine excretion to fluorine in the drinking water is shown by data in table 3. The consumption of fluoride waters by one of us (F. J. Mc.) produced an immediate proportional increase in urinary fluorine. During these field studies wherever

Table 1a

Fluorine present		Fluorine added		Fluorine found		Recovered
Micrograms	p. p. m.	Micrograms	p. p. m.	Micrograms	p. p. m.	Percent
40.0	0.4	20	0.2	70	0.7	117
30.0	.3	20	.2	45	.5	90
40.0	.4	50	.5	70	.7	78
30.0	.3	50	.5	87	.9	108
40.0	.4	80	.8	120	1.2	100
40.0	.4	100	1.0	150	1.5	107
30.0	.3	100	1.0	117	1.2	92
40.0	.4	150	1.5	180	1.8	95
40.0	.4	200	2.0	220	2.2	92
30.0	.3	200	2.0	241	2.4	104

specimens were collected the fluorine concentration of urine specimens of this individual followed closely the fluorine contained in the local drinking water.

As shown in tables 1 and 2, individual urine specimens were pooled to provide an analytical sample for fluorine determination. A total of 114 comparisons between fluoride domestic water and urinary fluorine, covering 13 levels of water-fluorine concentration, is available for study. Inspection of these data suggested that a logistic curve (*20*) would be most suited to describe graphically the water fluorine-urinary fluorine relation. Accordingly, using the equation $Y-d=\frac{K}{1+Ce^{rx}}$(*20*), the symmetric logistic curve shown in figure 2, was obtained. The data as used and the observed and predicted values for urinary fluorine are as follows:

Number of pooled urine analyses averaged	Fluorine in drinking water (p. p. m.)	Fluorine in urine (found) (p. p. m.)	Fluorine in urine (calculated) (p. p. m.)
35	0.0	0.340	0.348
8	.1	.375	.382
6	.2	.383	.420
1	.5	.600	.557
7	.7	.671	.660
8	1.0	.925	.875
1	1.3	1.000	1.127
8	1.7	1.516	1.534
14	1.9	1.814	1.763
2	2.0	1.900	1.883
22	3.8	3.739	3.785
3	4.5	4.166	4.143

Within the range of water-borne fluorine (0.0 to 4.5 p.p.m. F) the logistic curve appears particularly descriptive of these data. Absence of fluorine in water (X equals 0.0) gives values equalling 0.340 p.p.m. (observed) and 0.348 p.p.m. (calculated) for fluorine in urine, and the other observed and calculated values are in very close agreement. Within the range 1.0 p.p.m. to 4.0 p.p.m. fluorine in water the curve rises rapidly and begins to level off at slightly above 4.0 p.p.m. water-fluorine concentration. At higher levels of water fluorine, however, it is unquestioned that the urinary fluorine would continue to increase and, with additional data for waters above 4.5 p.p.m. fluorine the calculation of a logistic curve would be expected to give a higher value to the asymptote (*20*).

TABLE 2.—*Fluorine content of urine. Pooled specimens from high school boys exposed to drinking waters containing different quantities of fluorine*

Location	Date	Fluorine in water (p. p. m.)[1]	Fluorine in urine (p. p. m.)	Number of specimens pooled	Comment
Amarillo, Tex	*1943* July 17	4.3-5.1	4.6	26	Boys aged 16 to 18. 3 to 17 years in Amarillo.
Lubbock, Tex	July 14	3.8	3.5	15	Boys aged 16 to 18. 11 years in Lubbock.
	July 14	3.8	4.2	11	Boys aged 16 to 18. 1 to 5 years in Lubbock.
	July 14	3.8	3.8	26	Boys aged 16 to 18. 6 to 18 years in Lubbock.
Galesburg, Ill	Oct. 20	1.9	2.0	17	Boys aged 15 to 17 who had lived practically their entire lives in Galesburg.
	Oct. 20	1.9	1.5	9	
	Oct. 20	1.9	1.7	17	
	Oct. 20	1.9	2.0	15	
	Oct. 21	1.9	2.1	16	
	Oct. 21	1.9	2.0	18	
	Oct. 21	1.9	1.6	15	
	Oct. 28	1.9	1.8	25	Boys aged 13 to 16. Entire life spent in Galesburg.
	Oct. 28	1.9	1.2	21	
	Oct. 28	1.9	2.2	20	
Monmouth, Ill	Nov. 1	1.7	1.4	14	Boys aged 15 to 17. Entire life in Monmouth.
	Nov. 2	1.7	1.5	18	
	Oct. 29	1.7	1.7	20	Boys aged 14 to 17. Entire life spent in Monmouth.
	Oct. 29	1.7	1.4	15	
	Nov. 1	1.7	1.3	20	Boys aged 15 to 17. Lifetime in Monmouth or Monmouth area.[2]
	Nov. 1	1.7	1.1	23	
	Nov. 2	1.7	1.2	21	
	Nov. 2	1.7	1.1	25	
Aurora, Ill	Nov. 4	1.0	.8	25	Boys aged 15 to 17. Practically entire life spent in Aurora.
	Nov. 4	1.0	.9	25	
	Nov. 4	1.0	.9	25	
	Nov. 5	1.0	1.1	28	
	Nov. 5	1.0	.9	25	
	Nov. 5	1.0	.7	25	
	Nov. 5	1.0	1.0	20	
Elgin, Ill	Nov. 8	.7	.6	21	Boys aged 15 to 17. Practically entire life spent in Elgin.
	Nov. 8	.7	.4	23	
	Nov. 9	.7	.7	20	
	Nov. 9	.7	.6	24	
	Nov. 9	.7	.7	22	
	Nov. 10	.7	.9	24	
	Nov. 10	.7	.8	24	
Oklahoma City, Okla	July 22	.5	.6	26	Boys aged 16 to 17. 12 years in Oklahoma City.
Quincy, Ill	Oct. 25	.1	.6	16	Boys aged 15 to 17. Lifetime residence in Quincy.
	Oct. 25	.1	.4	11	
	Oct. 25	.1	.2	20	
	Oct. 26	.1	.2	14	
	Oct. 26	.1	.2	24	
Waukegan, Ill	Nov. 12	0	.4	20	Boys aged 15 to 17. Lifetime residence in Waukegan.
	Nov. 12	0	.6	22	
	Nov. 15	0	.5	20	
	Nov. 15	0	.4	25	
	Nov. 15	0	.4	17	
	Nov. 16	0	.3	17	
	Nov. 16	0	.3	21	
Little Rock, Ark	July 27	0	.3	26	Boys aged 16 to 18. 12 years in Little Rock.
Washington, D. C	*1944* Jan. 4	0	.3	22	Boys aged 15.[3]
	Jan. 5	0	.2	22	Boys aged 16.[3]
	Jan. 6	0	.2	23	Boys aged 17.[3]

[1] These figures are for samples of water taken from the high school tap at the time the urine specimens were obtained.

[2] Several of the boys in these groups lived outside the city limits and their drinking water exposures were not ascertained.

[3] These boys had spent their entire lives in Washington, D. C.

The association shown between water and urinary fluorine is strikingly similar to the relation between water fluorine and mottled enamel as observed by Dean (*3*). The logistic curve appears to describe adequately both mottled enamel and urine fluorine as related to fluorine in drinking water. It is to be noted, however, that the logistic curve which would appear to describe Dean's data showing the relation between fluorine in drinking water and index of dental fluorosis (fig. 2 in Dean's article (*3*)) applies to values of X (water fluorine) ranging from 0.0 to 14 p.p.m. There is every indication in Dean's data also that the maximum mottled enamel effect (index of dental fluorosis) is reached at about 6 p.p.m. water fluorine. The logistic curve for urinary fluorine concentration (fig. 2), on the other hand, applies only to a range of 0.0 to 4.5 p.p.m. fluorine in drinking water and is not intended to describe urinary-fluorine concentrations beyond 4.5 p.p.m. of water fluorine.

Without quantitative ingestion and excretion data it is obviously impossible to assess exactly the relations between total fluorine ingested from drinking water and food and the accompanying total fluorine elimination in urine, feces, and perhaps sweat. At the same time, in view of the large number of urine specimens represented and the wide variety of conditions surrounding the eating and drinking habits of these individuals, important deductions regarding the ingestion and metabolism of fluorine from food and drinking water seem justified.

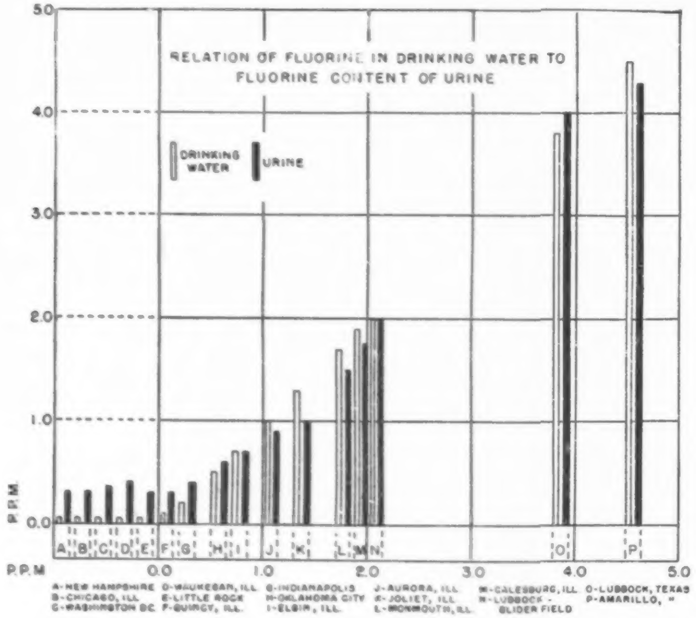

Figure 1

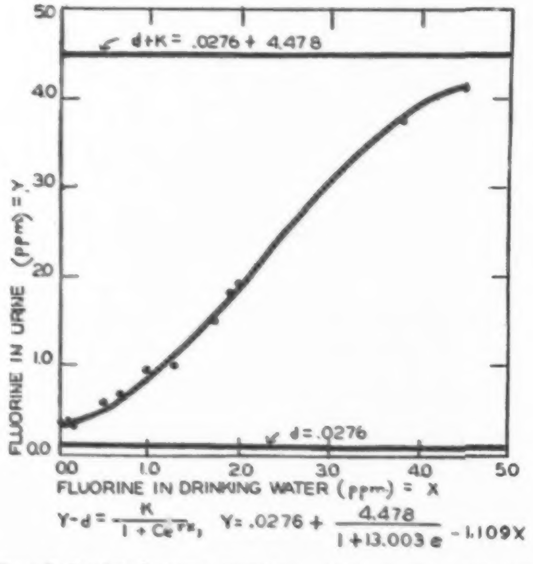

Figure 2.—*The relationship between fluorine in drinking water and fluorine in urine fitted with a symmetric logistic curve for the interval $x=0.0$ to $x=4.5$.*

First, it may be pointed out that the pronounced increases of fluorine in urine which accompany these exposures to fluorine in drinking water provide additional evidence that fluoride domestic waters are to be regarded as the most important as well as the most universal source of fluorine in human diets. The close correlation between urinary fluorine and domestic-water fluorine lends strong support to this conclusion. It is of interest to note also the uniformity in the urinary-fluorine figures for areas as widely separated as New Hampshire, Washington, D.C., Waukegan and Chicago, Ill., Quincy, Ill., Indianapolis, Ind., and Little Rock, Ark. (tables 1 and 2). A very uniform quantity of fluorine appears to be absorbed daily from foods as ingested by individuals residing in these several nonfluoride localities. These data suggest that the content of fluorine in the average human diet, exclusive of drinking water is remarkably uniform regardless of the locality. It may be suggested that this is indicative also that fluorine in food produce is quite uniform, regardless of the conditions under which the food is grown or produced.

The urinary fluorine data also seem to be in accord with previous estimates of total fluorine in the average diet exclusive of drinking water. Although analytical fluorine data on foods still lack the accuracy desirable for final conclusions, 0.2 to 0.5 p.p.m. or less fluorine seems to be a reasonably good estimate to apply to most fresh foods (*18*). Teas and seafoods in general contain unusual quantities of fluorine (teas 40 to 60 p.p.m., seafoods 2 to 12 p.p.m.), but it does not appear that these foods contribute significantly to the average daily fluorine ingested from food alone. The average daily diet has been estimated to contain 0.3 to 0.5 mg. fluorine (*18*). Numerous analyses of the daily diet of an adult man showed on the average 0.45 mg. fluorine present daily, exclusive of water drinking per se (*11*). This individual was residing in Cincinnati, Ohio, a nonfluoride area. As of November 1939, the Cincinnati water supply contained less than 0.2 p.p.m. fluorine (*19*). Another estimate, based on analyses of meals served to the staff of the Minnesota General Hospital, Minneapolis, Minn., indicates a daily intake of 0.27 to 0.32 mg. fluorine "from sources other than water" (*21*).

Urinary fluorine in concentrations equaling 0.3 to 0.5 p.p.m. (tables 1, 2, and 3) will account for approximately 0.3 to 0.5 mg. fluorine eliminated daily in the urine alone, assuming 1,000 ml. as an average daily volume of urine. In support of this estimate it may be noted that Brun, Buchwald, and Roholm (*13*) found 0.92 mg. to be the average quantity of fluorine in 24-hour urine specimens of 30 hospital patients aged 2 months to 70 years. The analytical figures for these daily specimens aver-

TABLE 3.—*Fluorine content of urine of one individual (F. J. Mc.). Residence in Washington, D.C., versus residence in Lubbock, Tex., and Galesburg, Monmouth, Aurora, Elgin, and Quincy, Ill.*

Location	Date (1943)	Fluorine in drinking water (p. p. m.)	Fluorine in urine (p. p. m.)	Comment
Washington, D. C.	Jan. 12	0.0	0.4	24-hour collection.
	Jan. 13	0	.2	
	Jan. 14	0	.3	
	June 29	0	.4	
	June 30	0	.3	
	July 1	0	.4	
Lubbock, Tex.	July 6	3.8	3.9	First 18 hours in Lubbock.
	July 7	3.8	3.9	Second 24 hours in Lubbock.
	July 8	3.8	4.0	Third 24 hours in Lubbock.
	July 9	3.8	3.5	Fourth 24 hours in Lubbock.
	July 11	3.8	3.8	Sixth day in Lubbock, night urine.
	July 13	3.8	3.5	Eighth day in Lubbock, night urine.
	July 15	3.8	3.6	Tenth day in Lubbock, night urine.
	July 17	2.8	3.5	Twelfth day in Lubbock, night urine.
Galesburg, Ill.	Oct. 21	1.9	1.8	First day in Galesburg, night urine.
	Oct. 22	1.9	1.8	Second day in Galesburg, night urine.
	Oct. 23	1.9	1.3	Third day in Galesburg, night urine.
	Oct. 24	1.9	2.4	Fourth day in Galesburg, night urine.
Quincy, Ill.	Oct. 25	.1	.5	First day in Quincy, night urine.
	Oct. 26	.1	.4	Second day in Quincy, night urine.
	Oct. 27	.1	.5	Third day in Quincy, night urine.
Galesburg, Ill.	Oct. 28	1.9	1.7	First day in Galesburg, night urine.
	Oct. 31	1.9	1.1	Fourth day in Galesburg, night urine.
Monmouth, Ill.	Nov. 2	1.7	1.0	Second day in Monmouth, night urine.
	Nov. 3	1.7	2.1	Third day in Monmouth, night urine.
Aurora, Ill.	Nov. 6	1.0	1.1	Third day in Aurora, night urine.
Elgin, Ill.	Nov. 10	.7	.7	Fourth day in Elgin, night urine.

TABLE 4.—*Fluorine content of urine. Relation to fluorine in drinking water. Summary of data appearing in tables 1 and 2*

Location	Fluorine in water (p. p. m.)	Fluorine in urine (p. p. m.)	Number of specimens represented	Comment
Amarillo, Tex.	4.7	4.6	26	High-school boys, aged 16 to 18.
	4.7	4.0	42	Selectees, Lubbock induction center.
Air Field, Lubbock, Tex.	5.1	4.0	50	Servicemen stationed at Air Field.
Lubbock, Tex.	3.8	3.8	62	High-school boys, aged 16 to 18.
	5.8	4.2	21	Texas Technical College men, aged 17 to 21.
	5.8	3.7	108	Selectees, Lubbock induction center.
Glider Field, Lubbock, Tex.	2.0	2.0	16	Servicemen stationed at Glider Field.
Galesburg, Ill.	1.9	1.8	173	High-school boys, aged 15 to 17.
Monmouth, Ill.	1.7	1.5	87	High-school boys, aged 15 to 17.
Joliet, Ill.	1.3	1.0	21	Selectees, Chicago induction center.
Aurora, Ill.	1.0	.9	173	High-school boys, aged 15 to 17.
Elgin, Ill.	.7	.7	158	High-school boys, aged 15 to 17.
Rural central Indiana	[1].5	.7	109	Selectees, Indianapolis, Ind., induction center.
Oklahoma City, Okla.	.5	.6	26	High-school boys, aged 16 to 18.
Indianapolis, Ind.	.2	.4	121	Selectees, Indianapolis induction center.
Chicago, Ill.	0	.3	60	Selectees, Chicago induction center.
Quincy, Ill.	.1	.3	85	High-school boys, aged 15 to 17.
Washington, D. C.	0	.4	202	Selectees, Fort Myer, Va., induction center.
	0	.3	67	High-school boys, aged 15 to 17.
Little Rock, Ark.	0	.3	26	High-school boys, aged 15 to 18.
Waukegan, Ill.	0	.4	142	High-school boys, aged 15 to 17.
New Hampshire	0	.3	174	Selectees, Manchester induction center.

[1] Estimated average for fluorine in waters in this area.

aged 0.80 p.p.m. fluorine. During the preparation of this manuscript, E. J. Largent and I. F. Ferneau (J. Indust. Hyg. and Toxicol., *26*: 113 (April 1944) presented evidence that spot urine specimens will give analytical fluorine figures almost identical with the fluorine analysis for the corresponding total 24-hour urine excretion. It may be suggested, therefore, that 0.3 to 0.5 mg. fluorine appears to be a reasonably good estimate of total daily fluorine eliminated in the average 24-hour urinary excretion, as applied to individuals 15 to 17 years old or older residing in nonfluoride areas.

Fluorine balance studies reported by Machle, Scott, and Largent (*11*) are of interest in these regards as showing that fluorine in food equaling about 0.3 to 0.6 mg. fluorine ingested daily, is largely eliminated from the body. Approximately 88 percent of the total fluorine ingested appeared in the urine, about 8 percent being excreted in the feces. These data (*11*) also are cited by these workers as emphasizing the significance of the urinary fluorine figure as an approximate index of the level of ingestion and elimination of fluorine as applied to the average adult.

The importance of water-borne fluorine as a source of fluorine in average diets has been strongly implied in previous epidemiological studies relative to water-borne endemic dental fluorosis (*3*), as well as in epidemiological studies showing the striking relation of fluorine in drinking water to dental caries (*4, 5*). As noted above, Dean (*4*) has failed to find the "fluoride intake from sources other than domestic water" sufficient "to produce even the faintest signs of dental fluorosis in as little as one percent" of a group of 2,042 white public school children using fluoride-free Lake Michigan water (*4*). The epidemiological surveys relative to water fluoride-dental caries relations, demonstrate a remarkable relation between fluorine in domestic water and dental caries experience in 12- to 14-year age groups exposed to these waters throughout life. This relation is maintained regardless of possible differences in local food conditions and thus appears to be an effect of water borne fluorine alone, over and above food fluorine. In all these epidemiological dental studies the evidence has been indicative (and now seems supported by urinary-fluorine data) that the predominant source of additional fluorine associated with dental effects of fluorine is the domestic water supply. There is little or no acceptable evidence that food produced in fluoride areas contains increased quantities of fluorine. It seems worth emphasizing that fluorine provided by sources other than drinking water in the average diet is a quite uniform quantity approximating 0.3 to 0.5 mg. daily. This much fluorine in the daily diet seems secondary in importance, both as to quantity and physiological effects, in comparison with the fluorine provided by fluoride domestic water supplies indigenous to the United States and other parts of the world. Insofar as the most significant source is the drinking water, fluorine appears to be a unique dietary constituent.

A second important implication surrounding the urinary fluorine figures relates to the daily accumulation of fluorine in skeletal tissue which might accompany exposure to fluorine in drinking water. It is strongly implied by these urinary fluorine data that fluorine present in drinking water in concentrations up to at least 4.0 to 5.0 p.p.m., when ingested by men and boys of these age groups, is largely eliminated in the urine. There is no reason to anticipate a sex difference in these regards, so it may be presumed that an efficient elimination of fluorine is characteristic of individuals in this age group, regardless of sex, exposed to these concentrations of fluorine in drinking water. It is not to be inferred, however, that these data indicate that storage of fluorine does not occur to any extent from these low concentrations of water-borne fluorine. This conclusion, as noted above, can be justified only by controlled balance experiments.

The hazard of cumulative fluorosis from water-borne fluorine in these concentrations may be minimized also by the following observations: (a) Fluorine is not an essential element but in small quantities appears to be a harmless constituent of all skeletal tissue. Trace quantities of fluorine in foods are a source of this fluorine. (b) According to experimental evidence fluorine may accumulate in skeletal tissue to an appreciable extent without injury. A tenfold increase in fluorine content of bones of experimental animals above an assumed normal of about 200 p.p.m. has been suggested by Peirce (*22*) as commensurate with absence of manifest morphological change in skeletal tissue. This is a highly tentative figure and may be too high. The tolerance of human skeletal tissue in terms of fluorine storage cannot be exactly stated at this time. (c) Results reported by Lawrenz, Mitchell, and Ruth (*23*) indicate that "growing rats adapt themselves to the continuous ingestion of low levels of fluorine by excreting greater and greater proportions of the ingested fluorine in feces and urine. This adaptation involves the excretory capacity of both kidney and intestine, that of the latter to a somewhat greater extent" (*23*). Fluorine in diets fed these rats ranged from 4 to 12.5 p.p.m. There is other evidence indicating that when increasing quantities of fluorine were ingested by a dog (*24*) and growing rats (*25*) a much larger percent of the quantity ingested was eliminated from the body. Other experiments by Lawrenz, Mitchell, and Ruth (*26*) also suggest an increased efficiency in fluorine elimination in rats, as associated with lengthening periods of fluorine feeding. (d) Mobilization of fluorine from skeletal tissue (*13*) seems to be a normal and perhaps a very important phase of fluorine metabolism. This defense mechanism has an analogy in the mobilization of lead from skeletal tissue. It may be a highly significant action connected with the defense of the organism against skeletal storage of toxic quantities of fluorine.

Excessive fluorine ingestion, however, under any circumstance, increases the fluorine stores of the body, and the tolerance level of fluorine per se in the skeletal tissue may be exceeded. Toxic bone fluorosis and accompanying morphologic effects will probably appear. However, systemic evidence of fluorine toxicosis related to skeletal storage of fluorine has not been reported as associated with the domestic use of fluoride waters most common in the United States. This subject had had very little study in fluoride areas of this country. It would appear, however, in the light of the urinary fluorine data available, that cumulative storage of fluorine as related to these low concentrations of fluorine in domestic waters is not sufficient to cause serious concern. As related to these low fluoride domestic waters, this form of fluorine toxicosis seems unlikely to become an endemic health problem.

Finally, it may be noted that fluorine in urines where 0.0 to 0.2 p.p.m. fluorine occurred in the drinking water averages 0.3 to 0.5 p.p.m. fluorine according to our data. This is considerably less fluorine than Machle's reported mean of 1.07 ± 0.02 p.p.m. fluorine in urines of 101 subjects selected at random (*9*). Although the residences of these individuals as reported by Machle (*9*), do not indicate an exposure to fluorine from local water supplies, there is no assurance of this fact. For comparison with our data it may be recalled also that Brun, Buchwald, and Roholm (*13*) found an average of 0.80 p.p.m. fluorine in 24-hour urine specimens of 30 hospital patients. The fluorine exposures of these patients also are not stated.

SUMMARY

The fluorine content of urine specimens of a large number of men and boys has been studied in relation to fluorine in their domestic water supplies. Where domestic waters are free of fluorine, the fluorine present in urine averages 0.3 to 0.5 p.p.m. An increase of fluorine in urine was associated with the use of domestic waters containing as little as 0.5 p.p.m. fluorine. Fluorine in urine specimens continues strikingly proportional to the fluorine content of the drinking water through the range of 0.5 to 5.1 p.p.m. fluorine in the domestic water. The results appear to furnish additional evidence of the importance of water-borne fluorine as a source of fluorine in human diets. The data agree with previous epidemiological studies which have demonstrated a striking relation between fluorine in communal water supplies and dental health, including reduced incidence of dental caries in 12- to 14-year-old children.

The close correlation between fluorine in drinking water and fluorine content of urine suggests that the presumed hazard of cumulative toxic bone-fluorosis surrounding certain water-borne sources of fluorine in the United States is greatly reduced by this relationship. An efficient urinary elimination of fluorine appears to be characteristic of individuals residing in certain fluoride areas of the United States, where the drinking water contains 0.5 to 5.0 p.p.m. fluorine. The metabolism of fluorine under these conditions seems to be a normal function of the human body and seems characterized by a condition approaching metabolic equilibrium, at least in the adult organism.

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PERMISSION FOR PUBLICATION

Permission for publication of the data obtained at armed forces induction centers and from the Lubbock Air and Glider Fields has been granted by the Office of the Surgeon General, War Department, Washington, D.C.

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BALANCES OF FLUORINE INGESTED FROM VARIOUS SOURCES IN FOOD AND WATER BY FIVE YOUNG MEN (Excretion of Fluorine Through the Skin)

F. J. McClure, H. H. Mitchell, T. S. Hamilton and C. A. Kinser

Ingestion of fluoride compounds in abnormal quantities generally results in extensive storage of fluoride in skeletal tissues. Bone abnormalities and systemic illness may occur when fluorine tolerance levels are greatly exceeded. Fluorine in certain domestic water supplies (*1, 2, 3*) and possibly as a foreign element on sprayed fruits (*4*), and fluorine contacts in certain industrial operations (*5*), are sufficient to suggest localized fluorine exposures hazardous to the public health. The hazard of endemic dental fluorosis (mottled enamel) limits the safe concentration of fluorine in a domestic water used throughout formative tooth life, to 1.0–1.5 p.p.m. fluorine (*3*). Public exposure to fluorine via domestic water containing 1.0 p.p.m. fluorine also may become more wide spread, if a proposed program of direct fluorination of drinking water for the partial control of dental caries, proves effective (*6, 7, 8*). The average daily diet exclusive of drinking water contains 0.2–0.5 mg. fluorine (*9, 10, 11*).

Clarification of fluorine public health hazards may be furthered by several procedures. One consists in observation of physiological effects by epidemiological methods applied to populations now exposed to fluoride domestic waters (*12, 13, 14, 15, 16, 17, 18*). Group studies also include clinical observation in connection with industrial fluorine exposures (*19, 20, 21*). A second procedure, followed in this experiment, studies fluorine ingestion and retention via controlled balance-studies on human subjects (*11, 22*). Numerous experimental animal and laboratory studies also are concerned with both acute and chronic fluorine toxicosis (*19, 23, 24, 25, 26, 27*).

Because of the unusual degree to which fluorine may become cumulative, more than ordinary significance is attached to evidence concerning its retention and elimination. Chronic cumulative toxic effects of fluorine may be predicted to a large extent by the quantity of fluorine which the body regularly retains (*19, 23, 24, 28*).

A daily intake of 0.5–1.5 mg. fluorine seems to be eliminated practically 100 percent by the adult organism (*11*). The addition of fluorides to an adult's diet (furnishing 6.0 mg. fluorine daily) has resulted, however, in positive daily balances of fluorine as follows: From sodium fluoride, 3.86 mg.; from calcium fluoride in solution, 3.81 mg.; from calcium fluoride (solid) in food, 2.21 mg.; from bone meal, 1.24 mg.; and from cryolite, 2.42 mg. (*22*).

In a recent epidemiological study McClure and Kinser (*18*) found that fluorine concentration of spot urine specimens has a strikingly close correlation with the fluorine concentration in domestic water, through the range of 0.5 to 4.5 p.p.m. fluorine in the drinking water. While quantitative relations of intake and output are lacking in this study, the results suggest strongly that the kidney is a remarkably efficient organ in eliminating fluorine ingested at those levels. It seems a notable observation (*18*) that in spite of the marked affinity of skeletal tissues for fluorine, the kidney excretes fluorine when as little as 0.5 p.p.m. fluorine is present in drinking water. Largely because of this active excretory kidney function the ingestion of domestic water fluorine in small quantities seems characterized by a condition approaching very near complete fluorine equilibrium, at least in the adult organism.

The purpose of the experiment to be described in this paper was to study the metabolism in adult human subjects of fluorides ingested in different forms and in different amounts. Particular attention was given to paths of excretion including the skin, and to levels of intake at which appreciable fluorine retention occurred. Since the experiment was conducted to study the metabolism of other substances as well as fluorine, its general plan was adjusted to these other studies.

PLAN OF EXPERIMENT

Five young men aged 19–27 years, lived under strict supervision in a common domicile in Urbana, Illinois, and reported to the University of Illinois Nutrition Laboratory each weekday for metabolism studies. The men spent 8 hours each day of a 5-day test period in an experimental chamber designed to control environmental temperature and humidity. Two environmental conditions were maintained, one "comfortable" when the temperature was 84°–85° F., relative humidity 49 percent–52 percent, and the other "hot-moist," when the temperature was 100°–101° F. and relative humidity 66 percent–70 percent. Sweating was usually profuse during the first hours of "hot-moist" periods. During "comfortable" periods the men wore only shorts and usually the body remained somewhat moist but there was little or no visible sweat.

The dermal excretion of fluorine which was an important phase of this study, was determined by thorough washing of the body prior to entrance into the experimental chamber, the collection of all sweat running from the body during the experimental exposure, in pans or in prepared cheesecloths, and a final thorough body washing at the termination of the exposure period. Tests indicated the methods used accounted for well over 90 percent of the sweat excreted. Owing to the necessity for large volumes of wash-water, the dermal excretions particularly during "comfortable" periods, were highly diluted. On Saturday morning, however, undiluted sweat was collected for analysis after a thorough body washing.

Fluorine supplements provided 3.0 mg. fluorine daily with the exception of two periods when bone meal furnished 1.5 mg. fluorine daily. The supplements were weighed into gelatin capsules, each capsule containing one-third of the daily allotment, i.e., 1.0 mg. fluorine. The contents of one capsule were sprinkled over the food at each meal, and the capsule itself then swallowed. Table 1 shows the schedule of fluorine supplemental additions, and the environmental conditions maintained in the experimental chamber.

Experimental study periods as a rule lasted 5 days, i.e., Monday through Friday, preceded by a preperiod on Saturday and Sunday. No body excretions were collected on these 2-day preperiods. Urine was collected for 24 hour intervals during all actual tests periods. When complete fluorine balances were determined, all food was weighed and an aliquot taken for analysis. Daily fecal excretions were collected, dried at 70° C. ground finely, and composited for each subject and for each five day period. Charcoal was used to separate the feces of each period. Urine and perspiration samples were preserved with toluene. Food aliquots were kept in the cold prior to analysis.

The quantity and variety of foods eaten during complete balance periods are illustrated by foods consumed at different times by subject "E". This information appears in table 2. The same foods were eaten by the other subjects but the quantities varied slightly. Fluorine in these mixed diets averaged 0.30 to 0.50 p.p.m. although high protein diets contained 0.70 to 0.90 p.p.m. fluorine. The diets were prepared with Urbana water which contained 0.30 p.p.m. fluorine which may have contributed slightly to the fluorine in cooked foods (*9, 29*). These men ate above average quantities of food providing 0.50 to 0.90 mg. fluorine daily, which is somewhat more than is expected in the average diet (*9, 10, 11*). Fluorine ingested in food as determined quantitatively in 12 periods was used to estimate food-fluorine ingested in other periods, there being no radical change in diets during such periods.

Determination of Fluorine.—The determination of fluorine followed the procedure proposed originally by Willard and Winter (*30*) and modified by Armstrong (*31*). Interference of chlorine was eliminated by use of silver perchlorate added to the distilling flask as proposed by Eberz, Lamb, and Lachele (*32*). The procedure for urine analysis has been reported previously in detail (*18, 33*). This procedure was also used for sweat and saliva analyses. Fresh food samples weighing 100 grams and dry feces samples of 5–10 grams each were taken for analysis. These were made slightly alkaline with 0.1 N. sodium hydroxide, 2.5 ml. of 5 percent magnesium acetate added, the sample dried and ashed at 500° C. Ashing of these samples is not altogether satisfactory. A pure white ash was not obtained prior to placing in the distilling flask. There appears to be no danger in distilling such samples from 60 percent perchloric acid. Titration of the distillate followed the usual procedure.

RESULTS OF EXPERIMENT

Quantitative data relative to fluorine ingestion and excretion are available from 28 periods during which usually four young adult men were studied (in four periods only three men were under

TABLE 1.—*Schedul: of fluorine balance studies*

PERIOD	CONDITIONS IN EXPERIMENTAL CHAMBER	FLUORINE EXPOSURES			
		Subject "[illegible]" or "F"	Subject "C"	Subject "D"	Subject "E"
11 12	comfortable hot-moist	Galesburg water	Galesburg water	Sodium Fluoride in water	Sodium Fluoride in water
13 14	hot-moist comfortable	Sodium Fluoride in water	Sodium Fluoride in water	Galesburg water	Galesburg water
15 16	comfortable hot-moist	— —	Galesburg water	Calcium Fluoride in water	Calcium Fluoride in water
17* 18*	hot-moist comfortable	— —	Calcium Fluoride in water	Galesburg water	Galesburg water
19* 20*	comfortable hot-moist	— (Subject "F")	Sodium Fluoride in food	Sodium Fluoride in food	Sodium Fluoride in food
21 22	hot-moist comfortable	Calcium Fluoride in food	Calcium Fluoride in food	Calcium Fluoride in food	Calcium Fluoride in food
23 24*	comfortable hot-moist	Bone Meal (1.5 mg. F daily)	Bone Meal (1.5 mg. F daily)	Bone Meal (1.5 mg. F daily)	Bone Meal (1.5 mg. F daily)
25* 27*	hot-moist comfortable	Bone Meal (3.0 mg. F daily)	Bone Meal (3.0 mg. F daily)	Bone Meal (3.0 mg. F daily)	Bone Meal (3.0 mg. F daily)
28* 29	comfortable hot-moist	Sodium Fluoride in water	Galesburg water	Galesburg water	Sodium Fluoride in water
30 31	comfortable hot-moist	Galesburg water	Sodium Fluoride in water	Sodium Fluoride in water	Galesburg water
33* 34*	hot-moist comfortable	Calcium Fluoride in food	Mineral cryolite in food	Mineral cryolite in food	Calcium Fluoride in food
35* 36	hot-moist comfortable	Mineral cryolite in food	Calcium Fluoride in food	Calcium Fluoride in food	Mineral cryolite in food
37 38	hot-moist hot-moist	Mineral cryolite in food	Synthetic cryolite in food	Synthetic cryolite in food	Mineral cryolite in food
39 40*	hot-moist comfortable	(none) "	(none) "	(none) "	(none) "

* Complete fluorine balances were determined during these periods. In other periods only fluorine in the urine and skin secretions, supplementary dietary fluorine, and fluorine ingested in drinking water were measured.

TABLE 2.—*Composition of diets—example of daily quantities ingested by subject "E"*

PERIODS	17, 18, 19, 20 (HIGH PROTEIN DIET)	24, 25, 27, 28, 40 (NORMAL DIET)	33, 34, 35 (LOW CALCIUM DIET)
Oatmeal, gm.	200	200	—
Orange, gm.	75	75	—
Egg, gm.	52	—	35
Prunes, gm.	—	—	70
Cream of wheat, gm.	—	—	10
Apple, gm.	98	83	—
Cheese, gm.	48	15	—
Meat sandwich, gm.	50	—	—
Cookies, gm.	16	40	70
Potatoes, gm.	100	175	250
Meat loaf, gm.	320	140	125
Corn, gm.	90	—	—
Celery, gm.	26	30	—
Apple sauce, gm.	80	—	—
Gravy, gm.	80	80	—
Pudding, gm.	140	200	—
Butter, gm.	15	15	30
Sandwich butter, gm.	12	10	—
Bread, gm.	165	165	290
Lunch meat, gm.	—	35	—
Beans, gm.	—	100	200
Carrots, gm.	—	30	—
Minced ham, gm.	—	—	35
Cream, gm.	—	—	40
Peanut butter, gm.	—	—	25
Apple butter, gm.	—	—	40
Cabbage, raw, gm.	—	—	40
Jello, gm.	—	—	15
Milk cc.	500	500	—
Tea, cc.	—	—	200
Average F (ppm.) in diet.	0.80	0.30	0.50
Average F (ppm.) in milk.	0.15	0.11	—
Tea as drunk, F (ppm).	—	—	0.50

observation). In addition to fluorine in supplements (table 1), an appreciable quantity of fluorine was ingested via Urbana water drunk in particularly large quantities during "hot moist" periods. With the exception of periods 11 through 16, all special sources of water-borne fluorine were consumed in 1600 cc volumes of water daily, the remainder of the drinking water requirement for the day being Urbana water. When all drinking water was a fluoride water, i.e., periods 11 through 16, the daily fluorine intake in hot moist periods became quite excessive and variations from period to period were quite extreme. Data as shown in table 3, for periods 11 through 16, do not permit averaging period results because of this variable fluorine intake. The percent elimination of fluorine in these periods was quite variable also. In general, however, the results indicate that perhaps better than 80 percent of these quantities of fluorine ingested in drinking water, was being eliminated in urine and perspiration.

Fluorine elimination data shown in table 4 are also limited to urine and perspiration. It should be noted particularly that these average figures show that fluorine ingested as sodium fluoride in drinking water; fluorine in Galesburg, Ill., drinking water; and fluorine as sodium fluoride in food, were on the average about 84 percent eliminated during "comfortable" periods (80 percent during "hot moist" periods) via urine and perspiration. The less soluble fluorides, calcium fluoride (in food) bone meal and cryolite, were not eliminated in such high percents in urine and perspiration, owing to the fact that they probably were not as completely absorbed from the digestive tract. This relation of solubility to absorption is referred to later.

Data for periods during which complete balances of fluorine were determined (table 5, fig. 1), give consistent evidence of practically complete elimination of fluorine ingested in quantities up to 5.0 mg. fluorine daily. Only one, possibly significant, positive balance of fluorine (1.2 mg. daily) resulted. This occurred from the ingestion of calcium fluoride in food during a hot moist period, and total daily fluorine ingested was 6.0 mg. It should be noted in regard to all the actual balance figures (table 5 and fig. 1) that they are perhaps minimal values, since the dermal excretion was determined for only 8 hours of the experimental day. During the other 16 hours some actual sweating may have occurred especially during summer months when the men occasionally engaged in sports. In any case, however, any loss by insensible perspiration would continue.

With regard to dermal losses of fluorine during "comfortable" periods, it should be noted that during those 8 experimental hours the men were scantly clad and at times their skin was notably moist. It may be recalled that the temperature was kept at 84°–85° F. It seems unlikely therefore that when the body was fully clothed in a cooler environment, the hourly dermal loss of fluorine was proportional to the average dermal fluorine elimination which occurred during the 8-hour "comfortable" period.

Fluorine in Perspiration. — Machle, Scott, and Largent (*11*) found the mean fluoride concentration of perspiration to be 0.514–0.066 p.p.m., "under conditions of high fluoride intake." When there was no fluorine exposure "amounts of fluorine in sweat were too small for determination." These authors discuss the possibility that a "definite loss of fluoride must be considered" "under conditions of excessive sweating."

Samples of undiluted sweat obtained during fluorine exposure periods and also after terminating the fluoride regimen, contained fluorine varying from 0.2 p.p.m. up to 1.8 p.p.m. (see table 6). As suggested by Machle, Scott and Largent (*11*), there is evidence in our data also (table 6) that the concentration of fluorine in sweat is related to the quantity of fluorine ingested.

According to the plan of our experiment, sweat and body washings were combined so that the total dermal loss of fluorine (expressed as milligrams of fluorine) is the only information directly available from the "sweat-plus-body-washings" data (see tables 4 and 5). It is possible however to use the figures representing 8-hour dermal losses of fluorine in connection with 8-hour net losses in body weight, to calculate the approximate fluorine concentration of the dermal excretion. The net loss in body weight consists of water lost as sweat (sensible water) plus water vaporized from lungs and skin and also represents any change in weight due to difference in weight between oxygen consumed and carbon dioxide expired. The correction for this latter respiratory exchange was made on net loss figures. The environmental temperatures were such that water vaporized from the lungs may be regarded as very slight. This corrected net loss in weight, therefore, is a good estimate of undiluted sweat and sensible perspiration losses. In "hot moist" periods this loss is mostly in the form of visible sweat. It varies on the average from 4000 to 65000 grams for all subjects for an 8-hour period. In "comfortable" periods the net weight loss averages 400–1000 grams for an 8-hour period and represents generally insensible perspiration. The fluorine concentration of this body perspiration calculated by dividing average total milligrams of fluorine in "sweat and body washings," by the daily average net loss in body weight, is as follows:

	SUBJECT "C"	SUBJECT "D"	SUBJECT "E"	SUBJECT "F"
	F-ppm	*F-ppm*	*F-ppm*	*F-ppm*
Calculations based on 8 to 11, hot moist periods (largely visible sweat)........	0.31 ± .05	0.30 ± .07	0.28 ± .07	0.34 ± .09
Calculations based on 6 to 9, comfortable periods (insensible perspiration)........	1.18 ± .30	0.85 ± .23	1.37 ± .23	1.38 ± .32

Profuse sweating in "hot-moist" periods gives a dilute excretion (sweat) showing much less fluorine than perspiration evaporated from the skin in "comfortable" periods. These calculated results, in general, are similar to fluorine concentrations determined by analysis of undiluted sweats (table 6).

TABLE 3.—*Individual fluorine ingestion and elimination data for periods 11 through 16. These data are not included in average figures shown in tables 4 and 5*

PERIOD	SPECIAL FLUORIDE-WATER EXPOSURE (a)	CONDITIONS IN EXPERIMENTAL CHAMBER	DRINKING WATER-FLUORINE DAILY	FLUORINE ELIMINATION					
				24-hour urine		8-hour perspiration		Total	
				MG.	%	MG.	%	MG.	%
			SUBJECT "B"						
11	Galesburg	comfortable	6.7	4.4	65.7	0.9	13.4	5.3	79.1
12	Galesburg	hot-moist	8.2	5.1	62.2	2.0	24.4	7.1	86.6
13	NaF-Urbana	hot-moist	9.0	4.7	52.2	3.7	41.1	8.4	93.3
14	NaF-Urbana	comfortable	5.7	3.8	66.7	0.9	15.8	4.7	82.5
			SUBJECT "C"						
11	Galesburg	comfortable	6.3	4.2	66.7	1.2	19.0	5.4	85.7
12	Galesburg	hot-moist	8.7	4.6	52.9	1.9	21.8	6.5	74.7
13	NaF-Urbana	hot-moist	8.3	3.6	43.4	2.0	24.1	5.6	67.5
14	NaF-Urbana	comfortable	6.9	4.4	63.8	0.9	13.0	5.3	76.8
15	Galesburg	comfortable	4.2	2.9	69.0	1.0	23.8	3.9	92.9
16	Galesburg	hot-moist	10.1	3.7	36.6	1.1	10.9	4.8	47.5
			SUBJECT "D"						
11	NaF-Urbana	comfortable	8.4	5.3	63.1	1.5	17.9	6.8	81.0
12	NaF-Urbana	hot-moist	10.2	4.3	42.2	2.7	26.4	7.0	68.6
13	Galesburg	hot-moist	12.2	6.8	55.7	3.9	32.0	10.7	87.7
14	Galesburg	comfortable	5.0	4.3	86.0	0.7	14.0	5.0	100.0
15	CaF_2-Urbana	comfortable	5.0	1.9	38.0	1.0	20.0	2.9	58.0
16	CaF_2-Urbana	hot-moist	10.9	1.8	16.5	1.9	17.4	3.7	33.9
			SUBJECT "E"						
11	NaF-Urbana	comfortable	9.0	4.4	48.9	0.9	10.0	5.3	58.9
12	NaF-Urbana	hot-moist	11.5	4.7	40.9	3.9	33.9	8.6	74.8
13	Galesburg	hot-moist	9.5	4.0	42.1	3.8	40.0	7.8	82.1
14	Galesburg	comfortable	4.7	3.6	76.6	0.9	19.1	4.5	95.7
15	CaF_2-Urbana	comfortable	3.8	1.3	34.2	0.7	18.4	2.0	52.6
16	CaF_2-Urbana	hot-moist	13.3	1.9	14.3	2.0	15.0	3.9	29.3

(a) All the drinking water was either Galesburg water or a synthetic fluoride water.

Fluorine in Saliva.—A number of stimulated saliva specimens were analyzed for fluorine and the following results obtained: Subject "C", 0.28 p.p.m., 0.22 p.p.m., 0.22 p.p.m., and 0.10 p.p.m. Subject "D", 0.11 p.p.m.. Subject "E", 0.11 p.p.m., 0.07 p.p.m., 0.05 p.p.m., 0.08 p.p.m., and 0.09 p.p.m.

There is no evidence in these data of any relation between quantity of fluorine being ingested and the salivary fluorine concentration The results agree with previous salivary fluorine figures obtained by McClure (*34*).

DISCUSSION

Absorption of fluorine from various fluoride compounds even at low levels of intake, was influenced to the greatest extent by the solubility of the compounds. Data in these regards compare quite favorably with results obtained by Machle and Largent (*22*).

The quantity of supplemental fluorine was 6.0 mg. daily in Machle and Largent's experiments. In our experiments supplemental fluorine equaled 3.0 mg. daily with the exception that bone meal in one balance study furnished 1.5 mg. fluorine daily. Data from both "comfortable" and "hot-moist" periods were averaged in obtaining our figures for percent absorption, there being no evidence that environmental conditions affected fluorine absorption.

FLUORINE EXPOSURE	FLUORINE INGESTED DAILY	FLUORINE ABSORBED	OBSERVER
	mg.	%	
Galesburg	3.77	89.7	McClure, Mitchell, Hamilton, Kinser
NaF in water	3.69	86.5	McClure, Mitchell, Hamilton, Kinser
CaF_2 in solution	6.25	95.7	Machle and Largent
CaF_2 in water	4.24	82.5	McClure, Mitchell, Hamilton, Kinser
NaF in food	6.48	97.1	Machle and Largent
NaF in food	4.76	83.3	McClure, Mitchell, Hamilton, Kinser
CaF_2 solid	6.44	62.1	Machle and Largent
CaF_2 in food	5.34	68.9	McClure, Mitchell, Hamilton, Kinser
Bone Meal	6.31	36.8	Machle and Largent
Bone Meal	4.18	53.6	McClure, Mitchell, Hamilton, Kinser
Cryolite	6.61	77.0	Machle and Largent
Mineral cryolite	5.34	68.2	McClure, Mitchell, Hamilton, Kinser

A summary of Machle and Largent (*22*) and our data appears at the left.

Elimination of absorbed fluorine is distributed between urine and perspiration in accord with environmental conditions. The kidney excreted on the average 77 percent of the absorbed fluorine, the skin through insensible perspiration 24 percent, during "comfortable" periods. In four out of seven "comfortable" period averages better than 80 percent of the absorbed fluorine appeared in the urine. During "hot-moist" periods the kidney eliminated on the average 49 percent of the absorbed fluorine, while the sweat during the 8-hour period accounted for an average of 44 percent. The combined elimination of absorbed fluorine via urine and perspiration averages 101 percent for "comfortable" periods, 93 percent for "hot-moist" periods.

The absorption data give some further information on the question of the relative hygienic equivalent of fluorine ingested in food vs. fluorine ingested in water (*28, 35, 36*). With repsect to both sodium fluoride and calcium fluoride ingested in water vs. food, absorption from drinking water-ingestion is greater than food-ingestion by 3.2 percent in case of sodium fluoride and by as much as 13.6 percent in the case of calcium fluoride, (above data by McClure et al). It should be noted that this comparison of calcium fluoride in food and water is based on results for only one subject in one 5-day period. Machle and Largent found a 33.5 percent difference favoring greater absorption of water-borne calcium fluoride over food-borne calcium fluoride. These data suggest that the factor of solubility of the fluoride itself may affect this result, calcium fluoride being much more insoluble than sodium fluoride. However, a difference of 3.2 percent favoring water vs. food-ingestion of sodium fluoride is too small to do more than suggest a physiological difference, and if true would seem to have only slight practical hygienic importance at these levels of sodium fluoride intake.

Data showing the elimination of fluorine ingested as cryolite, both mineral and synthetic forms, suggest that fluorine in these quantities remaining as a spray residue on apples and pears, will be largely eliminated by the human adult organism. Complete fluorine balances were obtained for mineral cryolite only (table 5), the results showing no significant fluorine storage. Urine and sweat elimination of fluorine ingested as synthetic cryolite (tables 4 and 5) was similar to other ingested fluorine. It may be noted, however, that Lawrenz and Mitchell (*37*), found fluorine in synthetic cryolite appreciably more completely retained by growing rats than fluorine in mineral cryolite. The greater solubility of the synthetic cryolite may explain this difference.

From the point of view of actual conditions surrounding domestic water fluorine exposures as well as in certain industrial fluorine exposures, the evidence that there is a significant elimination of fluorine in perspiration is an important finding. In warm climates or in hot summer weather or where industrial workers sweat profusely, shift of body water losses from kidney to skin would seem to reduce fluorine elimination. The data which we have obtained relative to hot-moist environmental conditions, suggest that perspiration may account for appreciable losses of fluorine. Under this circumstance very nearly complete fluorine equilibrium was maintained in a hot moist environment through the combined action of kidney and skin excretion. In connection with the perspiration losses of "comfortable" periods particularly, it should be emphasized, that the fluorine elimination figures for those periods are regarded as greater than would occur from insensible perspiration in more temperate environments. On the other hand there seems evidence

TABLE 4.—*Fluorine (milligrams daily) ingested from various sources and eliminated in urine and perspiration. These are average figures for all periods except periods 11 through 16*

SPECIAL FLUORINE EXPOSURE	NO. OF SUBJECTS	NO. OF PERIODS	FLUORINE INGESTED				FLUORINE ELIMINATED					
			Food	Special Source	Urbana Water	Total	Urine (24-hour)		Perspiration (8-hour)		Total	
Comfortable Periods												
			mg.	mg.	mg.	mg.	mg.	%	mg.	%	mg.	%
Galesburg water	4	7	0.5	3.0	0.2	3.7	2.6	70.3	0.7	18.9	3.3	89.2
NaF-Urbana water	4	4	0.5	3.0	0.2	3.7	2.2	59.5	0.7	18.9	2.9	78.4
CaF_2-Urbana water	1	1	0.5	3.0	0.3	3.8	2.7	71.1	0.9	23.7	3.6	94.7
NaF in food	3	3	0.6	3.0	0.7	4.3	2.9	67.4	0.8	18.6	3.7	86.0
CaF_2 in food	4	8	0.7	3.0	0.6	4.3	1.7	39.5	1.0	23.3	2.7	62.8
Bone Meal (1.5 mg. F daily) in food	4	4	0.5	1.5	0.5	2.5	1.1	44.0	0.5	20.0	1.6	64.0
Bone Meal (3.0 mg. F daily) in food	4	4	0.4	3.0	0.5	3.8	1.4	36.8	0.5	13.2	1.9	50.0
Mineral cryolite in food	4	4	0.9	3.0	0.6	4.5	2.0	44.4	0.7	15.6	2.7	60.0
None	4	4	0.8	0.0	0.7	1.5	0.9	60.0	0.4	26.7	1.3	86.7
Hot-Moist Periods												
Galesburg water	6	6	0.5	3.0	1.7	5.2	2.4	46.2	2.0	38.5	4.4	84.6
NaF-Urbana water	4	4	0.5	3.0	1.7	5.2	2.3	44.2	2.0	38.5	4.2	80.8
CaF_2-Urbana water	1	1	0.5	3.0	1.1	4.6	2.0	43.5	1.6	34.8	3.6	78.3
NaF in food	3	3	0.5	3.0	1.7	5.2	2.8	53.8	1.3	25.0	4.1	78.8
CaF_2 in food	4	8	0.7	3.0	1.8	5.5	1.8	32.7	1.6	29.1	3.4	61.8
Bone Meal (1.5 mg. F daily) in food	4	4	0.6	1.5	1.5	3.6	1.3	36.1	1.3	36.1	2.6	72.2
Bone Meal (3.0 mg. F daily) in food	4	4	0.4	3.0	1.8	5.2	1.0	19.2	1.7	32.7	2.7	51.9
Mineral cryolite	4	6	0.9	3.0	2.0	5.9	1.7	28.8	1.6	27.1	3.3	55.9
Synthetic cryolite	4	4	0.9	3.0	1.8	5.7	1.7	29.8	1.4	24.6	3.1	54.4
None	4	4	0.8	0.0	1.8	2.6	1.4	53.8	1.2	46.2	2.5	96.2

in these results that insensible perspiration is a path of fluorine elimination.

The observation that these five young men remained in practically complete fluorine equilibrium while ingesting these quantities of fluorine is a finding of particular interest with respect to cumulative toxic fluorosis. It does not appear that any appreciable accumulation of fluorine resulted from continued exposures to quantities of fluorine not exceeding 4.0 to 5.0 mg. daily. Two exposures representing 6.0 mg. fluorine ingested daily produced positive balances of fluorine equal to +20.0 and +5.0 percent respectively (see table 5). The data suggest the limit of total fluorine which may be ingested daily without an appreciable hazard of body storage is of the order of 4.0 m.–5.0 mg. fluorine daily. Subjects "C", "D", and "E" and "F", aged 27, 26, 21, and 20 yrs., respectively averaged 69.2, 88.0, 79.3 and 68.4 kg. body weight respectively during March, April and May, 1944, i.e., weeks 17–32. Their height was as follows: "C" 165.1 cm., "D" 187.3 cm., "E" 172.1 cm. and "F" 163.8 cm. Ingestion of 5 mg. fluorine daily by these men represents the following milligrams of fluorine daily, per kilogram of body weight: Subject "C" 0.072 mg., "D" 0.057 mg., "E" 0.063 mg., "F" 0.073 mg.

It was observed by Machle and Largent (*22*) that storage of fluorine occurred when fluorine ingested equaled approximately 6.0 mg. daily. It may be noted that whereas Machle and Largent's results refer to 6.0 mg. additional fluorine daily, our balances apply to not more than 3.0 mg. of supplemental fluorine daily. Urbana drinking-water-fluorine and food-fluorine brought the total daily fluorine intake of our subjects up to as much as 6.0 mg. fluorine daily during a few periods. It should be noted also that Machle and Largent (*22*) did not account for dermal losses of fluorine.

At the conclusion of the experiments, subjects "C", "D", "E", and "F", who had been ingesting fluorine in these quantities for a period of 21–26 weeks, were given a physical examination for the purpose of detecting possible signs of fluorine injury. Dr. Issac Schour of the College of Dentistry, University of Illinois, made a thorough clinical and radiographic examination of the teeth and surrounding tissues. The results lead him to conclude: "There is no finding apparent which could be associated with the fluorine experiment." Urine examinations for albumin, sugar and blood were negative. Red blood cell counts all exceeded 5 million per cmm. The men were in good physical condition throughout the study, and had no unusual subjective complaints. There was no localized sweat dermatitis due to sweat containing these elevated amounts of fluorine. The men were genuinely interested and cooperative throughout the study.

SUMMARY

1. Five young adult men served as subjects for the study of balances of fluorine, resulting from supplements of fluorine in

Table 6.—*Fluorine content of undiluted sweat. These samples of undiluted sweat were collected between balance periods, i.e., on Saturday mornings*

SUBJECT	VOLUME OF SWEAT	FLUORINE IN SWEAT	FLUORINE EXPOSURE
	cc	ppm	
B	1040	1.8	Galesburg water (a)
D	2350	1.2	Na F- Urbana water (a)
E	4540	1.0	Na F- Urbana water (a)
C	—	0.8	Ca F_2 in food (b)
D	—	0.6	" " " "
E	—	0.8	" " " "
F	—	0.3	" " " "
C	—	0.7	Ca F_2 in water (b)
C	500	0.5	Na F in food (b)
D	500	0.9	" " " "
E	500	0.7	" " " "
C	1330	0.8	Galesburg Water (b)
D	1300	1.4	" "
D	—	0.5	" "
E	1560	0.8	" "
E	—	0.8	" "
F	1220	0.9	" "
C	2200	0.6	Sweat was collected
D	2470	0.5	one week after
E	2890	0.4	terminating fluoride
F	1860	0.6	regimen
C	2620	0.2	Sweat was collected
D	3490	0.3	two weeks after
E	2300	0.2	terminating fluoride
F	3480	0.2	regimen

Table 5.—*Daily eliminations and balances of fluorine ingested from various sources in food and drinking water*

SPECIAL FLUORINE EXPOSURE	NO. PERIODS	NO. SUBJECTS	FLUORINE INGESTED				FLUORINE ELIMINATED								FLUORINE BALANCE	
			Urbana water	Special source	Food	Total	Urine		Perspiration*		Feces		Total			
COMFORTABLE PERIODS																
			mg.	mg.	mg.	mg.	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
Galesburg water	4	3	0.2	3.0	0.5	3.7	2.6	70.3	0.7	18.9	0.4	10.8	3.7	100.0	0.0	0.0
NaF—Urbana water	2	2	0.3	3.0	0.4	3.7	2.2	59.5	0.7	18.9	0.5	13.5	3.3	89.2	+0.4	+10.8
CaF_2—Urbana water	1	1	0.3	3.0	0.5	3.8	2.7	71.1	0.9	23.7	0.6	15.8	4.2	110.5	−0.4	−10.5
NaF in food	3	3	0.7	3.0	0.6	4.3	2.9	67.4	0.8	18.6	0.7	16.3	4.4	102.3	−0.1	−2.3
CaF_2 in food	2	2	0.7	3.0	1.0	4.7	1.7	36.2	0.8	17.0	1.7	36.2	4.2	89.4	+0.5	+10.6
Bone Meal (3.0 mg. F daily) in food	4	4	0.5	3.0	0.4	3.9	1.4	35.9	0.5	12.8	2.2	56.4	4.1	105.1	−0.2	−5.1
Mineral cryolite in food	2	2	0.8	3.0	0.9	4.7	2.9	61.7	0.9	19.1	1.5	31.9	5.2	110.6	−0.5	−10.8
None	4	4	0.7	0.0	0.8	1.5	0.9	60.0	0.4	26.7	0.5	30.0	1.8	120.0	−0.3	−20.0
HOT-MOIST PERIODS																
Galesburg water	2	2	1.7	3.0	0.5	5.2	2.6	50.0	2.2	42.3	1.2	23.1	6.0	115.4	−0.8	−15.4
CaF_2 Urbana water	1	1	1.1	3.0	0.5	4.6	2.0	43.5	1.6	34.8	0.9	19.6	4.5	97.8	+0.1	+2.2
NaF in food	3	3	1.7	3.0	0.5	5.2	2.8	53.8	1.2	23.1	0.9	17.3	4.9	94.2	+0.3	+5.6
CaF_2 in food	4	4	2.1	3.0	0.9	6.0	1.8	30.0	1.4	23.3	1.6	26.7	4.8	80.0	+1.2	+20.0
Bone Meal (1.5 mg. F daily) in food	4	4	1.5	1.5	0.6	3.6	1.3	36.1	1.3	36.1	1.3	36.1	3.9	108.3	−0.3	−8.3
Bone Meal (3.0 mg. F daily) in food	4	4	1.8	3.0	0.4	5.2	1.0	19.2	1.7	32.7	2.3	44.2	5.0	96.2	+0.2	+3.8
Mineral cryolite in food	4	4	2.1	3.0	0.9	6.0	1.9	31.7	1.8	30.0	2.0	33.3	5.7	95.0	+0.3	+5.0

* During 8-hour experimental period.

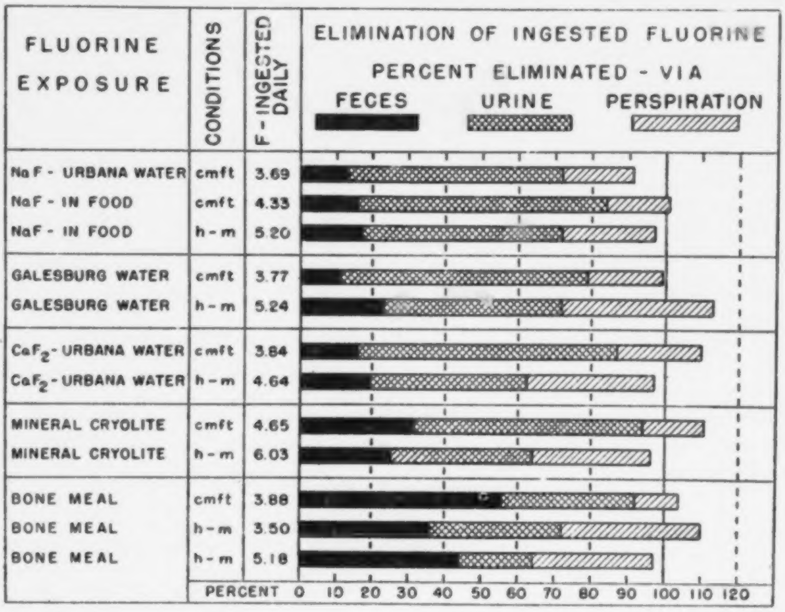

Figure 1.—*Percent of the ingested daily fluorine which was eliminated in the feces and urine daily and in perspiration excreted during an 8-hour period of the experimental day.*

various forms in food and drinking water. Balances were determined in five-day periods preceded by at least a two-day adjustment period. During 8 hours of each of the five days the men were kept in an atmosphere of controlled temperature and humidity. In "comfortable" periods, temperature was 84°–85° F., relative humidity 49–52 percent. In "hot-moist" periods temperature was 100°–101° F., relative humidity 66–70 percent. In addition to measuring body losses of fluorine in urine and feces, the loss of fluorine via insensible perspiration and sensible sweat was also determined.

2. Sweat was found to contain 0.3 to 1.8 p.p.m. fluorine during fluorine ingestion periods. During a postperiod 1 week after terminating the fluorine regimen, sweat contained 0.4–0.6 p.p.m. fluorine. During a following week, 2 weeks after terminating the fluorine regimen, sweat contained 0.2–0.3 p.p.m. fluorine. Sweat thus seems to be an important avenue for the elimination of fluorine. The insensible perspiration also may account for elimination of body fluorine.

3. The absorption of fluorine from bone meal, cryolite and calcium fluoride (in food) is appreciably less than from Galesburg domestic water or from sodium fluoride in food or water. It thus appears that absorption of these fluorine supplements even at these very low levels of intake, is largely dependent on the solubility of the supplement and in case of calcium fluoride on the method of administration, in solution or in solid form.

4. The elimination of absorbed fluorine via the urine and perspiration is practically complete when the quantities absorbed do not exceed 4.0–5.0 mg daily. There was no significant retention of fluorine in the bodies of these young adult men, when total daily fluorine ingested did not exceed 4.0 to 5.0 mg. daily. The data suggest that these may be the limits of fluorine which may be ingested daily without an appreciable hazard of body storage of fluorine.

5. The consistent evidence of practically complete fluorine equilibrium in these human subjects would seem to indicate also that exposure to domestic waters such as Galesburg, Ill., drinking water containing 1.8 to 1.9 p.p.m., fluorine or any drinking water which contributes an average of not more than 3.0 to 4.0 mg. fluorine daily to the ingesta, is not liable to create endemic cumulative toxic fluorosis. Similarly a domestic water to which sodium fluoride is added to give a fluorine content of 1.00 p.p.m. or less fluorine would not appear to create a public health hazard of cumulative toxic fluorosis.

6. Fluorine ingested in food in the form of bone meal, mineral cryolite, or calcium fluoride in quantities up to 3.0 mg. fluorine daily, was largely eliminated from the young adult body. Fluorine as cryolite in the form of spray residues on apples and pears, when ingested in quantities of 3.0 mg. or less fluorine daily, may be expected therefore not to entail a hazard of cumulative toxic fluorosis.

7. The results of this quantitative fluorine elimination study emphasize again the significance of the urinary excretion in maintaining a state of fluorine equilibrium in the human organism.

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This investigation was conducted in cooperation with the Division of Animal Nutrition, Department of Animal Husbandry, University of Illinois, Urbana, Ill.

FLUORINE IN DENTIN AND ENAMEL OF SOUND AND CARIOUS HUMAN TEETH

F. J. McClure

In a review of the literature in 1933, enamel, dentin, and whole teeth were reported to contain from 0.0 percent to 1.4 percent fluorine (*1*). The published results up to that time indicated the presence of 0.10 to 0.20 percent fluorine in enamel. Following 1933, the accuracy of the fluorine analytical procedure was improved but the quantity of data relative to dental tissues has remained very limited. Additional data on fluorine in human dentin and enamel are desirable, particularly since fluorine has gained much prominence in the prevention of dental caries (*2*). The published results on the fluorine content of teeth beginning with the year 1933 are summarized in table I, A and B.

While the data given in table I, A are insufficient to define the relation between fluorine content and mottled enamel diagnosis of individual teeth, as a general rule, exposure to fluoride waters, particularly during the formative period, results in more than normal percentages of fluorine being present in both dentin and enamel (see Ockerse (*6*), table I, A and B).

Fluorine determinations, however, are not consistent with respect to fluorine in sound versus carious teeth. The most extensive evidence on this question has been supplied by Armstrong and Brekhus (7) whose data are statistically significant and support the view that enamel of sound teeth may contain almost twice as much fluorine as enamel of carious teeth. These investigators found no differences in dentin of sound and carious teeth. Ockerse (*6*) reported identical mean values for fluorine in sound and carious enamel and no significant differences in dentin. Restarski (*8*), in heretofore unpublished analyses, obtained fluorine values which appear to be somewhat high for both sound and carious dentin and enamel. His data seem to imply a somewhat elevated fluorine exposure by the group of Samoan natives whose teeth he analyzed, and, as he reported (*14*), the Samoans have an unusually low caries index.

The mean fluorine content of enamel of nine sound teeth, according to Restarski's data, was 0.0150±0.0012 percent, whereas fluorine in the enamel of 14 carious teeth had a mean value of 0.0110±0.0010 percent. The difference between enamel of sound and carious teeth, with its probable error, is 0.0040±0.0015 percent fluorine, which is too small to be statistically significant. Aside from the data presented by Armstrong and Brekhus (7), therefore, there has been no evidence that enamel of carious and noncarious teeth differs in fluorine content. Furthermore, the analytical data thus far give no indication of how fluorine may vary from one type of tooth to another, or in the teeth of one individual as compared with those of another.

For obvious reasons the usual diagnosis of a tooth as carious upon the finding of cavities or fillings is not a criterion of active caries. An unfilled cavity is indicative of past or present caries activity; a filling is essentially a record of previous caries experience. Between the time of restoration or cessation of active caries and the date of tooth extraction, fluorine may have been acquired, that is, posteruptively (*15, 16*). It is important to note, therefore, that our designation as "carious" actually refers to teeth which had caries at a prior undetermined period and may or may not have been actively carious at the time of extraction.

The content of ash was determined as a routine part of the fluorine determination and is recorded in two of the tables. The ash data are consistent for all teeth, both sound and carious. The possibility that fluorine in the ash might be more revealing with regard to differences in fluorine content than fluorine in the unashed sample was given some consideration, but comparisons of fluorine values for ashed samples are no different from comparisons of fluorine in the unashed dentin or enamel. The major attention is given to fluorine in the unashed samples.

TABLE IA.—*Fluorine in sound and carious teeth not exposed to fluoride domestic waters (data publish after 1933)*

OBSERVER		DENTIN (% F)	ENAMEL (% F)	DESCRIPTION AND LOCATION
Boissevain and Drea[3]		0.0600	0.0250	Sound; New York City
Bowes and Murray[4]	Range	--	0.0-0.0200	Sound; London, Eng.
Bowes and Murray[5]	--	0.0250	0.0250	"Normal"
Ockerse[6]	Mean	0.0268	0.0153	Sound; non-F area; Knysna, S. Africa
	Range	(0.0200-0.0320)	(0.0130-0.0180)	
	Mean	0.0252	0.0153	Carious; non-F area; Knysna, S. Africa
	Range	(0.0146-0.0520)	(0.0070-0.0310)	
Armstrong and Brekhus[7]	Mean	--	0.0160	Sound; 36-year-old man
	Mean	--	0.0070	Carious
	Mean	--	0.0109	Sound; 28-year-old woman
	Mean	--	0.0063	Carious
	Mean	--	0.0112	Sound; sex and age unknown
	Mean	--	0.0067	Carious
	Mean	--	0.0114	Sound; mean of several individuals
	Mean	--	0.0068	Carious
	Mean	0.0169	0.0111	Sound
	Range	--	(0.0073-0.0167)	
	Mean	0.0158	0.0069	Carious
	Range	--	(0.0049-0.0087)	
Restarski[8]	Mean	0.0646	0.0150	Sound; teeth from Samoan natives
	Range	(0.0418-0.1071)	(0.0100-0.0243)	
	Mean	0.0423	0.0110	Carious
	Range	(0.0100-0.0903)	(0.0060-0.0246)	
Roholm[9]	Mean	0.0310*	0.0044*	Normal incisors
	Mean	0.0300	0.0057	Normal molars

*Roholm's figures refer to fluorine in ash.

TABLE IB.—*Fluorine in teeth exposed to fluoride (data published after 1933)*

OBSERVER		DENTIN	ENAMEL	DESCRIPTION AND LOCATION
Boissevain and Drea[3]	Mean	0.1120	0.0650	From fluoride area*
Bowes and Murray[5]	Mean	0.0730	0.0320	"Mottled teeth"; England
Armstrong and Brekhus[10]	Mean	--	0.0357	Mottled; severe
	Mean	--	0.0333	Mottled; severe
	Mean	--	0.0361	Mottled; moderate
	Mean	--	0.0343	Mottled; questionable
Sognnaes and Armstrong[11]	Mean	0.0196	0.0140	Deciduous; sound
	Mean	0.0270	0.0140	Permanent; carious or malposition From Tristan de Cunha†
Ockerse[6]	Mean	0.0755	0.0445	From Calvinia, S. Africa, a fluoride area‡
	Range	0.0130-0.1700	(0.0150-0.0750)	
Hamersma[12]	Mean	0.0456§	0.0177§	Teeth from 19-year-old boy living in Pretoria, S. Africa, a fluoride area
	Range	(0.0317-0.0680)	(0.0074-0.0285)	
Roholm[9]	Mean	0.0830§	0.0290§	Teeth from worker in cryolite (Na_3AlF_6) factory

*Colorado Springs drinking water which contains 2.5 to 2.6 ppm fluorine.

†A mild fluoride exposure from food is suggested in connection with this study.

‡Ockerse reported 1.5 ppm fluorine in drinking water from this area.[6] He reported also as much as 13.9 ppm fluorine in water from bore holes, used for drinking in some areas in South Africa.[13]

§Data refer to fluorine in ash.

Fluorine was determined in dentin and enamel according to Armstrong's modification (*17, 18*) of the Willard and Winter titration technique (*19*). Individual teeth was first cleaned and the crowns separated from the roots. All carious material was removed with dental burs. The crowns were pulverized to pass a 60-mesh sieve, extracted in a Soxhlet 8 hours with ether and 8 hours with alcohol, and dentin and enamel were seperated by the flotation process of Manly and Hodge (*20*).

The enamel and dentin samples were dried at 100° C. for one hour, and a sample for fluorine analysis was weighed into a tared platinum crucible. The sample was ashed at 550° C. for 3 hours and the weight of ash determined. The ash was transferred to a 50 cc. pyrex Claisen distilling flask containing a glass bead, and 10 cc. of 60 percent $HClO_4$ were added with about 10 cc. of water used for washing the ash from the crucible. Steam distillation was begun when the temperature in the distilling flask reached 138 to 140° C. and was continued until 150 cc. of distillate were collected in a receiving flask containing 5 cc. N/10 NaOH. The distillate was evaporated almost to dryness in a platinum dish, then made acid with N/10 HCl, and transferred to a 10 cc. volumetric flask. Three or four aliquots, consisting of one-fifth or one-tenth of the total, were titrated with standard thorium nitrate, after first buffering with one drop of the usual trichloracetate buffer and adding one drop of sodium alizarinsulfonate indicator. The mean of three or four aliquot titrations was used for the final calculations. In the majority of the analyses the quantity of determined fluorine was not less than 25.0 µg and rarely exceeded 100 to 150 micrograms. This necessitated starting with an analytical sample of enamel or dentin of the order of 0.2 to 0.5 gm., which in most instances made it necessary to pool enamel and dentin from several teeth. Teeth of a similar type were pooled in all but a very few instances. Some idea of the accuracy of the method may be gained from the analytical and recovery data shown in table II. Twenty-five and fifty micrograms of fluorine as sodium fluoride were added to dentin and enamel samples previously analyzed for fluorine. The micrograms and percent of fluorine recovered after the mean percent of fluorine present in the sample was deducted, are recorded in table II.

The error in the fluorine determination for dentin and enamel according to the data in table II is ±5.0 to 10.0 percent.

RESULTS

Any comparison of sound and carious teeth which is made between different individuals for fluorine differences is likely to introduce errors due to variations in the individual's fluorine exposures. Dietary fluorine which is influenced almost exclusively by the drinking-water fluorine (*22*) may be expected to affect the fluorine in teeth, particularly during pre-eruptive tooth life, and may also increase dental tissue fluorine posteruptively (*15, 16, 21*). In table III, this uncontrolled variation in fluorine exposures of sound and carious groups of teeth is disregarded and all the analytical data are averaged. In one group, there were 14 individuals who were continuous residents of McDuffie County, Ga., where the caries prevalence is somewhat low and there is no evidence of an unusual fluoride exposure. The 44 individuals listed as residing in northcentral Illinois constitute a variable group whose residence history was not established and remains quite uncertain. Although the teeth from these individuals were collected in a geographical area which has a history of moderate fluoride waters (1.5 to 2.5 p.p.m. F) (*2*), no elevation in enamel and dentin fluorine was disclosed by analysis. The data for 33 individuals were studied separately. These individuals constitute a random group representing individuals whose extractions were performed mostly in Washington, D.C. Their residence histories, so far as they could be obtained, varied greatly. The differences among the means of all the data for ash, fluorine, and fluorine in ash, shown in table III, have no statistical significance. These mean values were calculated from 132, 142, 150, and 187 individual analyses, representing 262 sound and 248 carious teeth from 91 individuals. The error due to nonrandom sampling differences (caused by variable fluorine exposures) in these group data may not be too significant in consideration of the relatively large number of individuals and teeth represented in the data. It should be noted also that none of these teeth had a pre-eruptive fluorine exposure large enough to produce mottled enamel. The extent of the variability in the data for different individuals is evident in the figures shown in table IV and may be explained as mentioned previously by the sensitivity of dental tissues to dietary fluorine.

Among the 33 individuals studied separately, there are 19 who provided both sound and carious dentin and 16 represented with both sound and carious enamel. A comparison of the fluorine in sound and carious dentin and enamel from these individuals is shown in table IV. The statistical comparison of the sound and carious "means" in table IV gains in significance because of the presence of teeth from the same individuals in both sound and carious categories. The mean difference between the amount of fluorine in sound and in carious dentin is +0.0005 ±0.0011 percent. Between sound and carious enamel the fluorine difference is —0.0003 ±0.0007 percent. Neither of these differences is statistically significant. When the individual differences were treated statistically by Student's method (*23*), the probability factors, 0.272 for sound versus carious dentin and 0.151 for sound versus carious enamel, were obtained (table IV). These factors give no indication that the differ-

Table II.—*Fluorine analyses of human dentin and enamel—recovery of added fluorine*

	Analysis 1	2	3	4	Mean
	Sample A (Sound Dentin)				
Per cent fluorine	.0228	.0230	.0231	--	.0230
Fluorine added µg	25.0	25.0	50.0	50.0	--
Fluorine recovered µg	27.7	29.0	52.7	42.6	--
Fluorine recovered %	110.8	116.0	105.4	85.2	104.4
	Sample B (Carious Dentin)				
Per cent fluorine	.0195	.0198	.0169	.0143	.0176
Fluorine added µg	25.0	25.0	25.0	50.0	--
Fluorine recovered µg	26.3	21.6	26.2	48.8	--
Fluorine recovered %	105.2	86.4	95.4	95.2	95.6
	Sample C (Carious Enamel)				
Per cent fluorine	.0083	.0081	.0080	.0094	.0085
Fluorine added µg	25.0	50.0	50.0	--	--
Fluorine recovered µg	20.5	44.2	47.6	--	--
Fluorine recovered %	82.0	88.4	95.2	--	88.5
	Sample D (Sound Enamel)				
Per cent fluorine	.0107	.0124	.0100	.0122	.0113
Fluorine added µg	25.0	50.0	50.0	--	--
Fluorine recovered µg	24.7	53.0	44.7	--	--
Fluorine recovered %	98.8	106.0	89.4	--	98.0
	Sample E (Sound Enamel)				
Per cent fluorine	.0120	.0114	--	--	.0117
Fluorine added µg	25.0	25.0	--	--	--
Fluorine recovered µg	24.1	25.8	--	--	--
Fluorine recovered %	96.4	103.2	--	--	99.8

Table III.—*Results of analysis of sound and carious teeth. Percent fluorine, ash, and fluorine in ash of enamel and dentin*

		Dentin								Enamel							
		Sound teeth				Carious teeth				Sound teeth				Carious teeth			
Number of sound teeth	Number of carious teeth	Number of analyses	Ash (%)	F (%)	F in ash (%)	Number of analyses	Ash (%)	F (%)	F in ash (%)	Number of analyses	Ash (%)	F (%)	F in ash (%)	Number of analyses	Ash (%)	F (%)	F in ash (%)
14 Individuals Who Were Continuous Residents of McDuffie City, Ga.—Nonfluoride Area																	
16	13	21	76.4	.0216	.0283	19	77.1	.0186	.0242	6	94.7	.0086	.0091	10	94.9	.0097	.0102
44 Individuals Residing in Northcentral Ill.—Moderate Fluoride Area																	
22	33	5	80.2	.0300	.0373	8	79.6	.0210	.0264	6	96.4	.0103	.0106	7	95.5	.0093	.0097
33 Individuals From Scattered Areas With No Indication of Fluorine Exposure																	
224	202	124	80.0	.0240	.0302	160	79.8	.0227	.0285	120	95.9	.0102	.0106	125	95.7	.0098	.0102
Total 262	248	150	--	--	--	187	--	--	--	132	--	--	--	142	--	--	--
Mean*		--	79.8	.0241	.0302	--	79.7	.0225	.0282	--	95.9	.0102	.0106	--	95.7	.0098	.0102
P.E. mean±		--	.3	.0010	.0012	--	.2	.0007	.0009	--	.1	.0004	.0004		.1	.0003	.0003
		Sound vs. Carious – differences								Sound vs. Carious – differences							
Ash		0.1 ± 0.4								0.2 ± 0.1							
Fluorine		0.0016 ± 0.0012								0.0004 ± 0.0005							
Fluorine in Ash		0.0020 ± 0.0015								0.0004 ± 0.0005							

*In calculation of this mean and its probable error, the result for each individual of the group of 33 individuals was used as a separate item, and the means of the other two groups constituted two other items. Among the group of 33 individuals, from 1 to 14 analyses per individual were averaged to obtain the individual items.

ences are significant. Only a probability factor of 0.05 or less justifies a significant interpretation (the odds are 1 to 20 or more that the result is not a chance happening). Therefore, in this comparison of sound and carious data (18 dentin and 16 enamel) from the same individuals, there is no indication that differences in fluorine content could be related to the carious or noncarious status of the tooth.

Different developmental and calcification characteristics of the various functional types of teeth (as well as variations in fluorine exposure) might introduce variations in the amount of fluorine acquired by the teeth, particularly as they develop and calcify. An attempt to eliminate this developmental variable is made in the analysis of the data shown in table V where the data compare carious and sound teeth of similar type from the same individual. For example, individual 1 had both sound and carious first incisors, or second incisors, or canines represented in the data. The differences (and their probable errors) in the fluorine found in the sound and carious enamel and dentin are tabulated (table V). These differences have no statistical significance; the probable error of each calculated difference is greater than the difference itself.

A study of the results with respect to teeth having different developmental characteristics was made also by means of the data shown in tables VI and VII. Here again, the uncontrolled, unknown variation in the individual's exposure to fluorine may have influenced the quantity of fluorine deposited, particularly during the formative period. The mean differences tested statistically according to the method of Student (*23*) gave probability factors permitting no interpretation of significance (table VI).

Table IV.—*Fluorine content of dentin and enamel of sound and carious teeth, comparisons made on the same individual*

		DENTIN			ENAMEL			RATIO DENTIN VS. ENAMEL	
INDIVIDUAL	AGE (YR.)	SOUND (% F)	CARIOUS (% F)	DIFFERENCE (% F)	SOUND (% F)	CARIOUS (% F)	DIFFERENCE (% F)	SOUND TEETH	CARIOUS TEETH
1	35	.0156	.0254	+.0098	.0070	.0109	+.0039	2.2	2.3
2	43	.0223	.0163	–.0060	.0073	.0054	–.0019	3.0	3.0
5	53	.0312	.0327	+.0015	--	--	--	--	--
6	35	.0133	.0119	–.0014	--	--	--	--	--
7	40	.0210	.0183	–.0027	.0096	.0068	–.0028	2.2	2.7
8	46	.0202	.0206	+.0004	0104	.0087	–.0017	1.9	2.4
9	26	.0319	.0294	–.0025	.0173	.0154	–.0019	1.8	1.9
10	23	.0246	.0270	+.0024	.0152	.0140	–.0012	1.6	1.9
11	45	.0205	.0169	–.0036	.0100	.0083	–.0017	2.1	2.0
12	--	.0256	.0216	–.0040	.0154	.0139	–.0015	1.7	1.6
17	--	.0175	.0235	+.0060	.0079	.0092	+.0013	2.2	2.6
20	60	.0191	.0205	+.0014	.0076	.0080	+.0004	2.5	2.6
21	39	.0219	.0201	–.0018	.0087	.0068	–.0019	2.5	3.0
23	56	.0156	.0194	+.0038	.0078	.0082	+.0004	2.0	2.4
24	--	.0204	.0210	+.0006	.0074	.0096	+.0022	2.8	2.2
25	42	.0313	.0311	–.0002	.0100	.0102	+.0002	3.1	3.1
26	51	.0240	.0222	–.0018	.0110	.0124	+.0014	2.2	1.8
28	--	.0168	.0197	+.0029	.0067	.0078	+.0011	2.5	2.5
31	47	.0136	.0177	+.0041	--	--	--	--	--
Mean		.0214	.0219	+.0005*	.0100	.0097	–.0003*	2.3	2.4
$P.E._m$		±.0008	±.0008	±.0011†	±.0005	±.0005	±.0007†	±.07	±.07

*Difference between means.

†P. E. difference.

Statistical analysis of differences according to Student[23]:

	FLUORINE SOUND VS. CARIOUS DENTIN	FLUORINE SOUND VS. CARIOUS ENAMEL
Mean difference	+.00005	–.00004
Standard deviation of difference	.00008	.00004
t value	.620	1.071
probability	.272	.151

Table V.—*Fluorine analyses of sound and carious teeth from the same individual (comparisons made between teeth of similar type)*

TYPE	NUMBER OF COMPARISONS	DENTIN SOUND (% F)	DENTIN CARIOUS (% F)	ENAMEL SOUND (% F)	ENAMEL CARIOUS (% F)
A	10	.0197	.0210	.0085	.0096
B	6	.0232	.0232	.0113	.0113
C	2	.0200	.0133	.0101	.0082
Mean P. E. mean		.0209 ±.0010	.0214 ±.0010	.0095 ±.0006	.0099 ±.0007
Difference		.0005		.0004	
P. E. difference		±.0014		±.0009	

Type A, First incisors, second incisors, canines; Type B, First premolars, second premolars; Type C, Second molars.

All of the data have been utilized in the preparation of table VII to study fluorine in the different anatomic types of teeth and in sound and carious teeth. Statistically the differences in data for sound and carious teeth in this table have no significance. The different teeth (combining all the sound and carious) vary in fluorine content between 0.0230 percent and 0.0247 percent in the dentin and between 0.0086 percent and 0.0120 percent in the enamel.

While the variation in fluorine in individual teeth is indicated to some extent by the data presented in table VII, some further idea as to the variations which may occur among individual teeth in the same mouth may be gained by inspection of the data shown in table VIII. In this table are presented the detailed results of analyses of all the teeth available from three individuals. The differences in the fluorine content of individual teeth from the same dentition may be considerable, as the data show for individual 13, particularly. All the teeth obtained from this individual were sound; the enamel fluorine varied from 0.0053 percent to 0.0123 percent, and the dentin varied from 0.0166 percent to 0.0327 percent fluorine. As a rule, the data for individual teeth were more uniform than that shown for individual 13.

DISCUSSION

One of the purposes of this study was to attempt to explain the role of fluorine as a caries-preventive element. One hypothesis receiving reasonably sound support ascribes the anticaries effects of fluoride domestic waters (*2*) and the effect of topically applied fluoride (*24, 25*) to the acquisition of an optimal quantity of fluorine by dental tissue. This hypothesis has been supported by the widely accepted but limited data of Armstrong and Brekhus (7) which, as quoted previously, show significant differences in the fluorine content of the enamel of sound versus carious teeth of three individuals. Other group-data presented by these same investigators reflect the same enamel differences, although no differences were observed in the dentin.

In the light of more extensive analytical data there seems considerable reason to believe that a difference in fluorine content of sound and carious enamel (and dentin) is not a general rule among most individual dentitions. The sound and carious enamel in the teeth of all of the 16 individuals showed no differences, and in all of the 19 individuals there were no consistent differences in the sound and carious dentin. Therefore, it does not appear that the caries history of one tooth as compared with another tooth in the same dentition was related to its fluorine content.

Analysis of the data in relation to variations in the fluorine content of different functional or anatomic types of teeth has brought to light no significant variations in either dentin or enamel. As in the comparisons of carious and sound teeth, it is interesting to note that the statistical results for both dentin and enamel indicate a similar pattern of uniformity. This relation is substantiated in the dentin to enamel ratios shown in table IV. The dentin seems to contain about 2.0 to 2.5 times as much fluorine as the enamel. In this connection, previous analyses of rats' molar and incisor teeth may be noted in which the dentin to enamel fluorine ratio was of the order of 2.5 to 3.0 for the incisors; and where fluorine had been acquired (even in varying quantities) posteruptively by the rats' molars, there was uniformly 3.5 to 4.0 times as much fluorine in the dentin as in the enamel (*16*). This relatively consistent relation of fluorine in dentin to fluorine in enamel in the case of rats' teeth was ascribed to an orderly systemic retention of fluorine by both dentin and enamel, which seemingly was unaffected by "oral surface absorption of fluorine." Human teeth-fluorine data also give good evidence of a relatively consistent ratio of fluorine in the dentin to fluorine in the enamel. This would not be the case in the carious tooth if, as the data of Armstrong and Brekhus (7) would indicate, there were less fluorine in the enamel but not in the dentin of the carious tooth. The weight of the evidence according to our data suggests, therefore, that all the teeth in a dentition generally respond with considerable uniformity to dietary-fluorine exposure, and fluorine in the dentin and enamel will be in a relatively uniform ratio. Variations among teeth appear to have no relation to the anatomic type or subsequent caries history.

While teeth studied individually in a single dentition may show no relation, therefore, between fluorine content and individual caries experience, such a finding is not contrary to the suggestion that the total caries experience of an individual may be greatly benefited by the presence of an over-all optimum quantity of fluorine in the dentin and enamel of all the teeth. It may be possible, therefore, to attribute the decrease in dental caries experience among individuals residing in areas of domestic fluoride waters (*2*) to an overall increase in fluorine in the dentin and enamel of all the teeth. In this way a critical concentration of fluorine in the dentin and enamel of the entire dentition may be expected to improve the total dental caries experience of the individual. In the future, our studies will attempt to relate fluorine in dentin and enamel to fluoride-domestic-water exposure, and these results may throw light on the relation of fluorine in dentin and enamel to the communal dental caries index. At the same time, additional data will be available to study sound and carious teeth as well as mottled teeth which have been exposed to domestic fluoride waters.

TABLE VI.—*Comparison of fluorine in different types of teeth from the same individual*

COMPARISONS	NUMBER OF INDIVIDUAL COMPARISONS	MEAN DIFFERENCE IN FLUORINE (%)	PROBABILITY FACTOR*
Canines vs. incisors			
Sound dentin	14	-.00012	.127
Sound enamel	13	-.00001	.461
Premolars vs. combined incisors and canines			
Sound dentin	10	-.00015	.073
Sound enamel	12	+.00009	.060
2nd molars vs. premolars			
Carious dentin	13	+.00002	.577
Carious enamel	12	-.00007	.073

*According to Student.[a]

TABLE VII.—*Fluorine in sound and carious teeth classified according to type*

	NUMBER OF SAMPLES	SOUND		NUMBER OF SAMPLES	CARIOUS		NUMBER OF SAMPLES	SOUND AND CARIOUS		SOUND AND CARIOUS	
		MEAN	P.E.		MEAN	P.E.		MEAN	P.E.	DIFFERENCE	P.E.
Fluorine in Dentin (Per Cent)											
Incisors	44	.0252	±.0009	28	.0231	±.0010	72	.0240	±.0007	.0021	±.0013
Canines	24	.0241	±.0011	19	.0219	±.0012	43	.0231	±.0008	.0022	±.0016
1st premolars	20	.0252	±.0011	18	.0212	±.0009	38	.0233	±.0008	.0040	±.0014
2nd premolars	19	.0276	±.0010	20	.0216	±.0009	39	.0245	±.0007	.0060	±.0013*
1st molars	10	.0265	±.0025	19	.0215	±.0010	29	.0232	±.0011	.0050	±.0027
2nd molars	13	.0245	±.0016	45	.0225	±.0008	58	.0230	±.0007	.0020	±.0018
3rd molars	18	.0276	±.0018	18	.0221	±.0011	34	.0247	±.0011	.0055	±.0021
Fluorine in Enamel (Per Cent)											
Incisors	22	.0118	±.0005	22	.0101	±.0005	44	.0110	±.0004	.0017	±.0007
Canines	21	.0093	±.0004	13	.0094	±.0004	34	.0093	±.0003	.0001	±.0005
1st premolars	15	.0108	±.0005	16	.0089	±.0005	31	.0098	±.0004	.0019	±.0007
2nd premolars	20	.0101	±.0005	17	.0115	±.0007	37	.0107	±.0004	.0014	±.0008
1st molars	9	.0074	±.0004	16	.0093	±.0005	25	.0086	±.0004	.0019	±.0006*
2nd molars	12	.0089	±.0005	35	.0108	±.0004	47	.0104	±.0004	.0010	±.0006
3rd molars	15	.0116	±.0006	16	.0124	±.0005	31	.0120	±.0004	.0008	±.0008

*Significant statistical differences are in excess of three times the probable error of the difference.

TABLE VIII.—*Fluorine in dentin and enamel (analytical data as obtained for three individuals)*

INDIVIDUAL 13			INDIVIDUAL 3			INDIVIDUAL 10		
TEETH EXTRACTED IN WASHINGTON, D. C.; AGE AND RESIDENCE UNKNOWN			46-YEAR-OLD MAN; CONTINUOUS RESIDENCE IN MCDUFFIE COUNTY, GA.			23-YEAR-OLD MAN; ENTIRE LIFE IN WASHINGTON, D. C. AREA		
TOOTH NUMBER*	SOUND		TOOTH NUMBER*	SOUND		TOOTH NUMBER*	CARIOUS	
	DENTIN (% F)	ENAMEL (% F)		DENTIN (% F)	ENAMEL (% F)		DENTIN (% F)	ENAMEL (% F)
1, 16	.0166	.0064	16	.0397	.0101	2	.0322	.0159
2, 15	.0240	.0114	3, 14	.0405	.0058	15	.0303	.0147
3, 14	.0228	.0053	4, 13, 20, 29	.0352	.0070	13	.0284	.0129
4, 13	.0270	.0103	5, 12, 21, 28	.0313	.0064	14	.0253	.0099
5, 12	.0284	.0120	6, 11, 22, 27	.0342	.0100	1, 3	.0248	.0132
6, 11	.0277	.0114	7, 10, 23	.0478	.0072	12	.0248	.0116
7, 10	.0293	.0117	8, 9, 24, 25	.0473	.0068	6, 11†	.0220	.0102
8, 9	.0308	.0114	32, 17	.0446	.0095	32	.0337	.0146
32, 17	.0233	.0100	31, 18	.0313	.0069	18	.0184	.0128
31, 18	.0217	.0064	30, 19	.0480	.0084	27, 22	.0249	.0127
30, 19	.0186	.0098						
29, 20	.0274	.0123						
28, 21	.0327	.0120						
26, 23	.0236	.0094						
Mean	.0253	.0100	Mean	.0400	.0078	Mean	.0265	.0129

*In most instances two or more teeth of similar type were pooled in order to obtain a quantity of analytical sample sufficient for one complete analysis. Teeth are numbered according to following formula: Upper right third molar (Number 1) to upper left third molar (Number 16); lower left third molar (Number 17) to lower right third molar.

†Sound.

SUMMARY

The fluorine content of human dentin and enamel has been studied by the analyses of teeth obtained from 33 individuals (studied separately) and from two groups of 14 and 44 individuals each. The crowns of 262 sound and 248 carious teeth were separated into dentin and enamel and a little over 600 fluorine determinations made on pooled or individual dentin and enamel samples. None of the teeth showed macroscopic fluorosis and, with the exception of the group of 44 individuals, there was no reason to suspect any unusual fluoride exposure. The ash in enamel averaged 95.8 percent; in dentin, 79.8 percent; and no differences were apparent between sound and carious teeth. The fluorine in enamel averaged 0.0100 percent and in dentin 0.0233 percent, and the differences between the enamel and the dentin of sound and carious teeth were not significant. Fluorine in the ash of enamel averaged 0.0104 percent and in the ash of dentin, 0.0292 percent. Differences between results for sound and carious teeth were not significant. A study of the teeth according to anatomic or functional type also did not reveal any significant differences in fluorine content.

An evaluation of the analytical data at present suggests that fluorine data for the individual teeth of a single dentition cannot be expected, as a general rule, to show consistently less fluorine in the enamel of the carious teeth than in the enamel of the sound teeth. In some individuals, fluorine in the enamel of the sound teeth alone may differ as much as the fluorine in enamel of sound versus carious teeth.

There is reason to believe that an unusual dietary (particularly water-fluoride) exposure during formative tooth life may increase the fluorine content of the entire dentition, including both dentin and enamel. An increase in fluorine in all the teeth may then account for a overall reduction in the dental caries experience. Under this circumstance it may be said that an optimum quantity of fluorine in the dentin and the enamel of the entire dentition is associated with a reduced incidence of dental caries. At the present time, data are not available to demonstrate this later hypothesis, but such an investigation is under way.

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FLUORINE, ASH, CALCIUM, AND PHOSPHORUS IN HUMAN TEETH

F. J. McClure

Although the actual relationship remains unknown, it is evident from previous publications (*1–5*), and it is apparent in the data of this report, that fluorine accumulates in human teeth when fluoride drinking waters are being ingested. When they develop in such an environment, human teeth may contain as much as 0.05 to 0.20 percent fluorine in the dentin and 0.03 to 0.10 percent in the enamel. These quantities of fluorine are characteristic, therefore, of severely and perhaps moderately mottled teeth. In general, human enamel formed under a nonfluoride environment will average approximately 0.010 percent fluorine and dentin will average 0.023 percent fluorine (*6, 7*).

Aside from fluorine, mottled (fluorosed) human teeth have not been studied very extensively for other chemical changes. Bowes and Murray (*2*) found no sigificant differences in the calcium, phosphorus, and magnesium content of mottled vs. nonmottled teeth. Other results, including iron, are likewise negative (*8*). Spectrographic analyses of human dentin and enamel reported by Lowater and Murray (*3*) indicated that "potassium was not found generally but was present in enamel and dentin of 'mottled teeth' and in fluoride-fed rats' teeth." The iron content was also diminished in the enamel of fluoride-fed rats. Fluorine varying from 0.029 percent to 0.095 percent in swine rations increased the total tooth fluorine up to 0.375 percent but chemical analyses revealed no concomitant effect on ash, calcium, phosphorus, magnesium, or carbon dioxide content (9). Rats' molar teeth also did not vary in ash, calcium, and phosphorus, although the tooth fluorine varied from 0.0310 percent up to 0.1842 percent (*10*). However, the incisor teeth of rats given rations containing 0.15 percent sodium fluoride in the diet decreased in total ash, even on a relatively high calcium intake (*11*). Fluorine in similarly excessive quantities (0.1 percent sodium fluoride in the ration) reduced ash and phosphorus, and increased slightly the calcium in rats' incisor teeth (*12*). Fluorine acquired by the enamel and dentin of dogs' teeth after tooth eruption, i.e., "secondary fluorine," (*13*) was not associated with any change of ash in the enamel or dentin (*14*).

The bones of animals and human beings generally are quite susceptible to an alteration in calcification as a result of fluoride ingestion (*9, 12, 15, 16*). Thus, 0.03 percent and 0.06 percent sodium fluoride in rats' diets increased the ash content of bones and decreased the calcium content of the ash (*15*). The level of calcium in rats' rations appears to be a predetermining factor in the effect of fluorine on bone ash (*11*). The classical studies by Roholm (*16*) established the effect of excessive fluorine on human bone calcification.

In the previous studies of the ash, calcium, and phosphorus in mottled vs. normal human teeth the variations in fluorine content have not been very pronounced (*2, 3*). Thus, the pooled mottled teeth analyzed by Bowes and Murray (*2*) and the spectrographic analyses of Lowater and Murray (*3*) were made on normal (London, England) enamel and dentin containing 0.025 percent fluorine vs. mottled enamel and dentin (from Maldon, England) containing 0.032 percent and 0.073 percent fluorine, respectively. The spectrographic studies by Boissevain and Drea (*1*) were made on normal enamel (0.0 percent fluorine) vs. mottled enamel (0.063 percent fluorine) and on normal dentin (0.068 percent fluorine) vs. fluorosed dentin (0.112 percent fluorine).

It was desirable, therefore, to accumulate additional data relative to the relation of dental fluorosis to calcification of dental tissues, which would represent wider variations of fluorine in the teeth and which would be related to the specific fluorine content of the teeth rather than to the mottled enamel diagnosis. It was sought to ascertain also the relation of a moderate or nonpathologic quantity of fluorine in dentin and enamel to the ash, calcium, and phosphorus content.

The human teeth analyzed in this study were extracted from individuals living in several fluoride and nonfluoride areas, all the highly fluorosed teeth, beyond reasonable doubt, having acquired fluorine by prolonged ingestion of fluoride drinking waters. Both sound and carious teeth were included, previous data (*17, 18, 19*), as well as our own data (table I), having shown no relation of calcium, phosphorus, and ash to caries experience. Similarly the age of the teeth was ignored since most previous data (*18, 19*) have shown (as do our own data in table I) no relation of age to ash, calcium, and phosphorus. There was no possibility of differentiating between fluorine acquired before vs. after the eruption of these teeth, although admittedly these two types of acquired dental fluorine, i.e., "primary" and "secondary," (*13*) might react differently.

The teeth were cleaned, all carious material removed, the crowns separated, dried, broken into small pieces, extracted with alcohol and ether, and pulverized to pass a 60-mesh sieve. The dentin and enamel were separated according to the Manly-Hodge technic (*20*) and ash, calcium, and phosphorus were determined by standard analytical methods (*21, 22*).

The data presented in table I conform with previous data (*16, 17, 18*) and indicate, as mentioned above, no differences in ash, calcium, and phosphorus in carious vs. noncarious teeth and no relation of ash, calcium, and phosphorus to tooth age.

In table II are the means of the analytical results arbitrarily grouped according to the fluorine conent of the dentin and enamel. As it appears from these data, there is no change in enamel ash, calcium, phosphorus, and the calcium-phosphorus ratio, although the mean enamel-fluorine varied from 0.006 percent to 0.049 percent. Although the mean fluorine in dentin varied from a low value of 0.012 percent up to 0.125 percent there was no change in the total calcium and phosphorus or the calcium-phosphorus ratio. There is the suggestion (table II), however, that the ash content of the highly fluorosed dentin is above normal. Comparing the mean ash figure, i.e., 80.1±0.668 percent ash in dentin (which averaged 0.125±0.0097 percent fluorine) with 77.6±0.345 percent ash in dentin (which contained 0.021±0.0007 percent fluorine), the difference is 2.5±0.751 percent which is statistically significant.

While a slight increase of the ash in dentin therefore may be a characteristic of moderate and severely mottled human teeth (0.030 to 0.100 percent or more fluorine), there is no evidence that lesser quantities of fluorine in the dentin will affect the ash or calcium and phosphorus content. Slight increases in enamel fluorine and dentin fluorine, however, are expected (although not demonstrated) to occur where drinking waters contain upwards of 1.0 to 1.5 p.p.m. fluorine. Under such fluoride drinking water exposures, the dental caries experience is reduced some 60 to 65 percent in the 12- to 14-year-old population (*23*). There is no explanation as to the mechanism of this anticaries effect of fluoride drinking waters. It would appear, however, from the data in table II that slight accumulations of fluorine in those teeth which are noncarious because of a fluoride water exposure do not alter the ash, calcium, or phosphorus content. These data furnish no indication, therefore, that the explanation of the fluorine anticaries

TABLE I.—*Ash, calcium, and phosphorus content of dentin and enamel related to caries*

	NO. OF TEETH	AGE (YR.)	ASH (%)	CA (%)	P (%)	CA:P (RATIO)
Enamel of sound teeth	30	45 ± 2.33*	95.1 ± .164	36.7 ± 251	17.5 ± .071	2.10 ± .019
Enamel of carious teeth	45	42 ± 2.15	94.6 ± .106	37.1 ± .116	17.4 ± .036	2.13 ± .069
Dentin of sound teeth	28	45 ± 2.33	77.2 ± .403	29.3 ± .182	14.0 ± .074	2.09 ± .011
Dentin of carious teeth	45	42 ± 2.15	76.3 ± .370	29.2 ± .180	14.0 ± .069	2.09 ± .008
Enamel aged 16.40 yr.	24	27 ± 1.32	94.6 ± .136	36.9 ± .169	17.4 ± .056	2.12 ± .010
Enamel aged 40-60 yr.	44	53 ± 0.84	94.6 ± .104	37.4 ± .101	17.4 ± .028	2.15 ± .006
Dentin aged 16-40 yr.	24	27 ± 1.32	76.5 ± .506	29.1 ± .199	14.1 ± .099	2.07 ± .011
Dentin aged 40-60 yr.	44	53 ± 0.84	76.3 ± .316	29.4 ± .184	14.0 ± .059	2.10 ± .008

$$^{*}\sigma_M = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

TABLE II.—*Ash, calcium, and phosphorus in dentin and enamel in relation to fluorine content*

NUMBER OF TEETH ANALYZED	RESULTS OF ANALYSIS OF DENTIN AND ENAMEL				
	F (%)	ASH (%)	CA (%)	P (%)	CA:P (RATIO)
	Fluorine in dentin = 0.007 – 0.014 per cent				
15	0.012 ± 0.0006*	77.4 ± 0.592	29.5 ± 0.225	14.1 ± 0.096	2.10 ± 0.016
	Fluorine in dentin = 0.015 – 0.030 per cent				
39	0.021 ± 0.0007	77.6 ± 0.345	29.5 ± 0.141	14.0 ± 0.073	2.11 ± 0.011
	Fluorine in dentin = 0.031 – 0.050 per cent				
9	0.038 ± 0.0020	76.9 ± 0.850	29.5 ± 0.336	13.9 ± 0.088	2.12 ± 0.017
	Fluorine in dentin = 0.076 – 0.213 per cent				
20	0.125 ± 0.0097	80.1 ± 0.668	29.1 ± 0.188	14.6 ± 0.191	2.01 ± 0.031
	Fluorine in enamel = 0.003 – 0.009 per cent				
22	0.006 ± 0.0004	95.4 ± 0.186	36.5 ± 0.281	17.5 ± 0.082	2.08 ± 0.020
	Fluorine in enamel = 0.010 – 0.025 per cent				
22	0.015 ± 0.0010	95.8 ± 0.131	37.1 ± 0.297	17.6 ± 0.095	2.11 ± 0.026
	Fluorine in enamel = 0.030 – 0.100 per cent				
19	0.049 ± 0.004	95.9 ± 0.233	36.3 ± 0.349	17.8 ± 0.129	2.05 ± 0.025

$$^{*}\sigma_M = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

effect is related to the total quantities of ash, calcium, and phosphorus or the calcium-phosphorus ratio of the enamel and dentin.

As has been pointed out previously (*2*) mottled enamel "is not due to diminished calcification." The data accumulated in this current study agree with this previous observation and also extend this conclusion to include teeth which may contain slightly increased quantities of fluorine but are nevertheless not mottled. Speculation continues none the less on the probable effect of fluorine ingestion on the type and physical properties of the apatite complex laid down in enamel and dentin during tooth calcification.

SUMMARY

1. The ash, calcium, and phosphorus content of human enamel and dentin did not vary with caries experience or age.

2. An eightfold increase in enamel fluorine was not related to any change in enamel ash, calcium, or phosphorus.

3. A tenfold increase in dentin fluorine was not related to any change in dentin, calcium, and phosphorus. There was a slight increase in the ash content of highly fluorosed dentin.

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FLUORINE IN HUMAN TEETH STUDIED IN RELATION TO FLUORINE IN THE DRINKING WATER

F. J. McClure and R. C. Likins

In a previous publication the senior author reported that there were no differences in the fluorine content of enamel and dentin of sound versus carious teeth and that fluorine differences between individual teeth would not explain the presence or absence of caries (*1*). At the same time it was suggested that there was reason to believe "that an unusual dietary (particularly water fluoride) exposure during the formative tooth life may increase the fluorine content of the entire dentition" and that such an increase in fluorine content might account for an overall reduction in the dental caries experience. A considerable quantity of data has now been accumulated which gives some insight into the relation of fluorine in drinking water to the fluorine in dentin and enamel of sound and carious teeth. These data are the first to be presented in an attempt to answer the question as to the effect of drinking water containing an optimal quantity of fluorine (i.e., 1.0 to 1.2 p.p.m. fluorine) on the fluorine in dentin and enamel of individuals continuously exposed to such a fluoride water.

In a previous publication (*1*) the data of other investigators relative to fluorine in human teeth were reviewed. Since that publication (June, 1948) two other contributions are known to have appeared in the literature (*2*, *3*). Harrison (*2*) found the mean fluorine content of New Zealand-born natives' teeth was 0.0042 percent for the enamel of 89 subjects and 0.0060 percent for the dentin of 8 subjects. "There was no significant difference between the fluorine content of the sound enamel of carious and noncarious teeth, all being low in this element." (*2*) New Zealand drinking waters contain 0.2 p.p.m. or less fluorine (North Island) (*4*) and less than 0.05 p.p.m. fluorine (South Island) (*5*). Fluorine in the drinking water of Switzerland as reported by Schmid (*3*) rarely exceeds 0.50 p.p.m. and most potable Swiss waters contain slightly less than 0.10 p.p.m. fluorine. Accordingly, fluorine in tooth-ash was found by Schmid (*3*) to be very low, i.e., rarely more than 0.010 percent and most of the results show 0.003 to 0.009 percent fluorine.

RESULTS

The procedures followed in the preparation and fluorine analysis of dentin and enamel are given in a previous publication (*1*). The results of our most recent analyses together with some results which have appeared previously (*1*) are presented in table I.

The teeth obtained from individuals living in McDuffie County, Ga., Washington, D.C., and vicinity, and Oslo, Norway, so far as was possible to ascertain, had been exposed to minimal quantities of water-borne fluorine. Among these three groups the average enamel-fluorine was 0.0092 percent, 0.0100 percent and 0.0079 percent and dentin-fluorine was 0.0201 percent, 0.0233 percent, and 0.0086 percent respectively. Individual results may vary considerably as shown by the maximum and minimum values in table I. Excepting the low dentin figure for teeth from Oslo, these average values are regarded as fairly indicative of the amount of fluorine which may be present in dentin and enamel of individuals normally exposed to drinking waters whose fluorine content is of the order of 0.0 p.p.m. to 0.3 p.p.m.

As noted in the footnote to table I, the teeth from the north central Illinois area were obtained from individuals of unestablished residence history as well as of uncertain water-fluoride exposures. Although collected in a geographical area which has a history of mottled enamel (*6*), there is little or no indication in these data of any elevation of fluorine in the dentin and enamel.

The data relative to teeth from Aurora, Ill., are particularly important since they relate to the effect of a fluoride drinking water which is caries preventive but will not produce any appreciable incidence of mottled enamel.

The Aurora data are broken down in table I, according to the age of the individual, but these figures give no evidence of differences among the age groups. The data indicate, however, that there is a general elevation of fluorine in both dentin and enamel when the drinking water contains 1.1 to 1.2 p.p.m. fluorine. The residence histories of these individuals were quite carefully checked and all the individuals are regarded as having had a continuous exposure to this fluoride water. Among these Aurora teeth there were 23 noncarious teeth which averaged 0.0403±0.0008 percent fluorine in the dentin and 0.0114±0.0001 percent fluorine in the enamel, and 78 carious teeth which averaged 0.0367±0.0003 percent fluorine in the dentin and 0.0147±0.0001 percent fluorine in the enamel. The number of analyses for sound teeth is limited and a comparison of sound versus carious teeth is of uncertain value. As these Aurora, Ill., data now stand there is no suggestion that the carious teeth contained less fluorine than the noncarious teeth.

The data respecting the effect of Galesburg, Ill., water (1.9 p.p.m. fluorine) are scanty indeed, owing to difficulties in obtaining teeth. Only a very few enamel analyses were obtained and are not reported. Most of these teeth were carious and it was often impossible to obtain sufficient enamel for analysis. The dentin-fluorine increased (average, 0.0425 percent) but not as much as might be anticipated from 1.9 p.p.m. fluorine in the drinking water.

The potable waters of the West Texas Panhandle area are consistently high in fluorine (2.5 to 5.0 p.p.m. F.). Exposure to these waters is very decidedly reflected in the fluorine results for dentin (0.0762 percent) and enamel (0.0345 percent) as shown in table I.

Campagnano di Roma, Italy, is a well-known mottled enamel area and the 12 teeth from this area showed mild to moderate mottling. The dentin- and enamel-fluorine, however, are much higher in these teeth than would be anticipated from the mottled enamel diagnosis and from the fluorine in the drinking water. It seems likely, therefore, that during some earlier period in the lives of these Italian natives their drinking water contained appreciably more than 3.5 p.p.m. of fluorine. Schour and Massler (*6*) also suggest that "a lowered nutritional status resulted in an increased prevalence and a higher degree of mottling in the teeth of children using a fluorine-containing communal water supply." Judging from our high fluorine results for these teeth from Italy, this might be true if water consumption was unusually high because of absence of milk from the children's diet.

The Chetopa, Kan., teeth were developed at a time when the water contained 7.6 p.p.m. of fluorine and the mottled enamel index was 3.2 and 100 percent of the population was affected (*7*). All of the seven noncarious teeth showed moderate to severe mottling.

Sufficient data have not been accumulated for a full-scale survey of the effect of a wide range in water-borne fluorine

Table I.—*Fluorine content of teeth from various geographical areas and fluoride-water exposures*

NUMBER OF INDIVIDUALS	GEOGRAPHICAL AREA AND TOOTH DESCRIPTION	FLUORINE IN DRINKING WATER* (PPM)	FLUORINE CONTENT OF					
			DENTIN			ENAMEL		
			MAX. (%)	MIN. (%)	AVE. (%)	MAX. (%)	MIN. (%)	AVE. (%)
9	McDuffie County, Ga.	0.1						
	16 noncarious teeth		0.0480	0.0099	0.0216	0.0220	0.0051	0.0086
	13 carious teeth		0.0544	0.0143	0.0186	0.0193	0.0040	0.0097
33	Wash., D. C., and vicinity	0.0 – 0.2						
	224 noncarious teeth		0.0482	0.0113	0.0240	0.0203	0.0046	0.0102
	202 carious teeth		0.0517	0.0085	0.0227	0.0220	0.0042	0.0098
9	Oslo, Norway	0.2 – 0.4						
	27 noncarious teeth		0.0126	0.0050	0.0086	0.0178	0.0025	0.0079
44	North central Ill.	--						
	22 noncarious teeth		0.0368	0.0107	0.0300	0.0132	0.0045	0.0103
	33 carious teeth		0.0521	0.0069	0.0210	0.0173	0.0037	0.0093
31	Aurora, Ill. Age 8-25	1.1 – 1.2						
	36 carious and noncarious teeth		0.0702	0.0125	0.0360	0.0253	0.0064	0.0135
12	Age 25-45							
	35 carious and noncarious teeth		0.0632	0.0216	0.0361	0.0221	0.0048	0.0142
17	Age over 45							
	30 carious and noncarious teeth		0.0706	0.0253	0.0140	0.0200	0.0096	0.0140
13	Galesburg, Ill.	1.9						
	29 carious and noncarious teeth		0.0692	0.0261	0.0425	--	--	--
--	West Texas Panhandle Area	2.5 – 5.0						
	21 carious and noncarious teeth		0.1033	0.0550	0.0762	0.0564	0.0149	0.0345
12	Campagnano di Roma, Italy	(3.5)						
	12 carious and noncarious teeth		0.2139	0.1123	0.1589	0.0772	0.0386	0.0599
--	Chetopa, Kan.	7.6						
	7 noncarious teeth		0.1814	0.0809	0.1277	0.1059	0.0384	0.0658

*The sources of the data on the fluorine content of these drinking waters are as follows:

(a) McDuffie County, Ga. Four samples from different areas in the county were obtained in January, 1945, and were analyzed by Dr. Elias Elvove, National Institutes of Health.

(b) Oslo, Norway. Ten samples of water were supplied by Dr. Guterom Toverud in June, 1948, and were analyzed by the senior author.

(c) Washington, D. C. Various samples of water were analyzed by Dr. Elias Elvove and the senior author during the years 1935 to 1948.

(d) North central Illinois. The waters from this area are quite variable—see Deatherage, C. F., Klassen, C. W., and Weart, J. G.: Fluoride and Mottled Enamel in Illinois, *Illinois D. J.* 8: 194, 1939. The uncertain and no doubt variable residence histories of these individuals does not permit any good estimate of their water fluoride exposures. The fluorine data on the teeth suggest, however, that the water-fluorine was probably not above 0.2 to 0.3 ppm.

(e) Aurora, Ill. See Reference 7.

(f) Galesburg, Ill. See Dean, H. T., Jay, Philip, Arnold, F. A., Jr.: McClure, F. J., and Elvove, Elias: Domestic Water and Dental Caries Including Certain Epidemiological Aspects of Oral L. Acidophilus, *Pub. Health Rep.* 54: 862, 1939.

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(h) Campagnano di Roma, Italy. One water sample was shipped from Italy in February, 1946, by Dr. Isaac Schour of the University of Illinois School of Dentistry and analyzed by Dr. Elias Elvove. This result (3.5 ppm) is lower than would be anticipated from the results of the teeth analyses. Visintin and Gandolfo, however, [Il fluro in natura come agente morbigeno (Nota 1. Le acque ed il suolo di Campagnano di Roma. Estratto Dai Rendiconti, Dell Istituto Superiore di Sanita. Roma, 22: 265, 1947] report only 0.61 to 1.17 ppm fluorine in six samples of drinking water from this area.

(i) Chetopa, Kan. See Reference 6.

on fluorine in teeth. A major difficulty in a study such as this is the necessity of ascertaining the individual's residence history before accepting teeth for analysis. The continuity of exposure (as well as the quantity of fluorine in the drinking water) is obviously a most important factor affecting the fluorine in teeth.

DISCUSSION

A number of reports appearing prior to this publication have shown that fluorine accumulates in dentin and enamel of mottled teeth, i.e., when there has been an exposure to a fluoride water for some considerable duration in the formative period of the teeth. These reports were reviewed in a previous publication (*1*). Little or no information was presented in these previous studies, however, in regard to the concentration of fluorine in the drinking water to which these teeth were exposed. In these previous data, as well as in our own data, the correlation between severity of mottling and the fluorine content was not significant. These data have established the fact, however, that fluorine does accumulate in abnormal quantities in mottled teeth.

The quantity of fluorine in dentin and enamel of teeth which are nonmottled but which have been exposed continuously to a drinking water containing 1.1 to 1.2 p.p.m. fluorine had not been studied prior to this report. It will be recalled that the dental caries experience among such teeth is about 65 percent below normal and mottled enamel is practically absent (*8*). Any attempt to explain this effect of fluorine on dental caries must take into account the fluorine in the enamel and dentin. In connection with the low incidence of dental caries in persons from Aurora, Ill., it was important, therefore, to ascertain the amount of fluorine in Aurora teeth as compared with the fluorine in "control" or "minimal-fluoride-exposure-teeth." It now seems apparent that 1.1 to 1.2 p.p.m. fluorine in drinking water does cause an appreciable increase of fluorine in the dentin and enamel of teeth continuously exposed to such drinking water. In enamel this increase amounts to about 30 percent and in the dentin to about 60 percent. These increases are based on an average of 0.0102±0.0004 percent fluorine in enamel of "control" or "nonfluoride teeth" (*1*) versus 0.0133±0.0001 percent fluorine in enamel of Aurora teeth and 0.0241±0.0010 percent fluorine in the dentin of "control" or "nonfluoride teeth" (*1*) versus 0.0385±0.0002 percent fluorine in the dentin of Aurora, Ill., teeth. The difference in enamel fluorine is 0.0031±0.0004 percent. The difference in dentin fluorine is 0.0144±0.0010 percent. Both differences are statistically significant.

The importance of these data, in connection with theoretical considerations of caries etiology, lies in the fact that a reduction in dental caries due to consumption of a near optimal concentration of fluorine in drinking water is directly associated with an increase of fluorine in dentin and enamel. Thus the role of fluorine, as deposited during formative tooth life, remains a prominent factor to be considered in solving the question of resistance to dental caries.

CONCLUSIONS

The teeth of individuals continuously exposed to drinking water containing 0.0 to 0.3 p.p.m. fluorine may be expected to contain approximately 0.0100 percent fluorine in the enamel and 0.0240 percent fluorine in the dentin. A near dental-optimal quantity of fluorine in Aurora, Ill., drinking water (i.e., 1.1 to 1.2 p.p.m.) resulted in 0.0133 percent fluorine in the enamel and 0.0385 percent fluorine in the dentin. These additional quantities of fluorine in dentin and enamel are associated with a marked reduction in the dental caries experience, but dental fluorosis is not an endemic health problem among such teeth. Data are presented to show the effect of other higher levels of fluorine in drinking water on the fluorine in human dentin and enamel.

The authors are indebted particularly to the dentists of Aurora, Ill., who cooperated in collecting Aurora teeth for analysis; to dentists in Galesburg, Ill., and the Texas Panhandle area; and to Dr. I. Schour of the University of Illinois School of Dentistry, for the teeth from Italy.

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EFFECT OF FLUORIDE IN DRINKING WATER ON THE OSSEOUS DEVELOPMENT OF THE HAND AND WRIST IN CHILDREN

By H. Berton McCauley and F. J. McClure

Numerous clinical, as well as experimental, studies have produced evidence that detrimental effects on skeletal tissues may result from prolonged exposure to large quantities of fluorides. Roholm (*1*) described the skeletal effects of an estimated exposure amounting to 25 to 30 mg. fluorine (F) inhaled daily for 9 years or longer as follows: "The pathological process may be characterized as a diffuse osteosclerosis in which the pathological formation of bone starts both in periosteum and in endosteum; compacta densifies and thickens; the spongiosa trabeculae thicken and fuse together. The medullary cavity decreases in diameter. There is considerable new formation of bone from periosteum, and ligaments that normally do not calcify, or only advance in age, undergo a considerable degree of calcification." Exposure to cryolite dust was the cause of the bone pathology observed by Roholm, as it was of that reported by Moller and Gudjonsson (*2*).

Prolonged exposure to fluoride in quantities over 10 p.p.m. F in drinking water has been reported responsible for chronic fluorosis characterized by rigidity in the spine, stiffness, and immobility of the joints, symptoms similar to those recorded by Roholm. These effects, attributed to excessive waterborne fluoride, have been observed endemically in the Madras Presidency (*3*) and the Punjab (*4*) of India and in the Pretoria district (*5*) of South Africa. As pointed out by McClure (*6*), not only is the fluoride content of the drinking water excessive in these areas, but also the hot climate increases water consumption, and low dietary and other living standards possibly aggravate the manifestations of skeletal abnormalities.

EXPOSURE IN THE UNITED STATES

In the United States, about 4.5 percent of the population is now using water which naturally contains 0.5 to 8.0 p.p.m. F. In addition, approximately 785 communities with a total population of about 15.5 million are (Nov. 1953) using water supplies in which the fluoride level is maintained within the range 0.7 to 1.5 p.p.m. F, by controlled fluoridation, for the reduction of dental caries. In excess of 1.5 p.p.m. F in drinking water, fluoride is responsible for the production of endemic dental fluorosis, or mottled enamel, in varying degrees of severity and incidence (*7*).

A reliable basis for the point of view that concentrations of fluoride in drinking water up to 1.5 p.p.m. are not a public health hazard is the evidence pertaining to urinary excretion. When the drinking water contains as much as 4.5 p.p.m. F, excretion is more than 90 percent effective (*6, 8, 9*). Such a highly efficient excretion largely negates the possibility of cumulative skeletal fluorosis from continuous exposure to drinking waters containing up to 4.5 p.p.m. F.

Further evidence of the harmlessness of low-fluoride water is available in observations relating specifically to the skeletal tissues. McClure (*10*) found that exposure to fluoride waters containing up to 4.5 p.p.m. F affected neither the bone-fracture experience nor the height and weight of high school boys and young men. A skeletal survey (*11*) of children and adults living in an area of endemic mottled enamel and exposed to drinking water containing 1.5 to 3.0 p.p.m. F yielded no radiological evidence of bone abnormality. Smith (*12*) also reported no changes in calcium and phosphorus metabolism in children with mottled enamel.

Indications that the fluorine concentration of the skeletal tissues increases gradually with age were found in an analysis of the ribs and vertebras of 158 persons with continuous lifelong residence in an area of relatively fluoride-free domestic water (*13*). It is probable that the fluorine in skeletal tissues can increase several times above average quantities without causing a deleterious skeletal effect (*6*).

In a comparison of medical histories and results of physical examinations, including complete X-rays, on adults who had lived 15 years or longer in Bartlett, Tex., where the drinking water contained 8.0 p.p.m. F, with similar information on adults living in Cameron, Tex., where the drinking water contained about 0.3 p.p.m. F, the only difference in the health status was shown by the X-rays. Among the Bartlett adults, 12 percent, all of whom were over 50 years of age and had used the Bartlett drinking water for more than 35 years, showed a somewhat coarser trabecular structure of the bones. These bone changes produced no effective signs or subjective symptoms related to the skeletal system and were regarded as having no general health significance (*14*).

The present study was organized to provide additional data on the skeletal effects of exposure to fluoride in drinking water in the United States. It was designed to determine specifically the effects of continuous exposure to drinking water containing 3.5 to 5.5 p.p.m. F on ossification and skeletal maturation as indicated by radiographs of the hand and wrist of children 7 through 14 years old.

RADIOGRAPHIC STUDIES OF BONE DEVELOPMENT

Because it is accessible and relatively convenient to radiograph, the hand has been the subject of frequent X-ray study since Roentgen's discovery. Ranke (*15*) in 1896, using wrist films, was apparently the first to witness skeletal growth. Pryor (*16*) in 1905 reported the earliest extensive roentgen-ray observations of bone growth and development in the hand.

Pryor (*16, 17*) studied more than 500 films of children from birth to 14 years of age and found a definite and uniform order of ossification of the carpal bones. Although the centers of calcification practically always appeared in the same order, the progress of ossification was subject to delay by ill health, metabolic disorder, interference with blood supply, accident, or disease. He discovered also that the skeleton of the growing girl is more mature than that of the boy of the same age and that the hand is a good index of ossification of the entire skeleton (*18*).

Skeletal Age

Comprehensive investigations by Flory (*19*), Todd (*20*), Greulich (*21*), and others have, in general, confirmed and amplified the observations of the early investigators and provided standards for the practical assessment of skeletal age by radiographic inspection of the hand. Ample evidence that the progress of development of the bones of the hand and wrist parallels that of the rest of the skeleton is provided in these and numerous other reports of the chronology of appearance and fusion of centers of ossification throughout the body. Todd (*20*) assessed skeletal maturity on the basis of X-ray findings in the hand, foot, elbow, knee, shoulder, and hip and decided that the hand was the most reliable single index.

The standards used to assess skeletal age in the present study are the composite record of skeletal development of Cleveland children from all grades of society, except the destitute, without regard to ethnic origin, stature, or weight (*20*). They represent large numbers of children radiographed at 6-month age intervals from birth through the 18th year and have been widely utilized in overall growth studies.

Ossification Index

Use of skeletal age in attempts to grade children for educational purposes caused investigators to seek a procedure for determining developmental status more objective than the inspectional technique. Baldwin (*22*) in 1921 was the first to report measurement of the carpal bones for this purpose, using a planimeter to ascertain the total area of their shadows on the X-ray picture. Later, Baldwin, Busby, and Garside (*23*) published a series of such measurements for school-age children, corrected for gross size by dividing the ossified area by the area of a rectangle of dimensions derived from two diameters of the wrist obtained with calipers. Similar quantitative indexes and norms of skeletal development were produced by Carter (*24*) and Kelly (*25*). The Carter (*24*) method, used in the present study, has been applied successfully in general growth (*19*) and nutritional (*26, 27*) problems in children.

STUDY AREAS

Amarillo and Lubbock, in western Texas, were selected for this study because they are the two largest communities in the United States having 3.5 p.p.m. F or more in their public water supplies. The populations are 74,000 and 71,000, respectively.

The fluoride content of the drinking water in Amarillo, according to frequent analyses by the city chemist, ranged from 3.6 p.p.m. F to 6.2 p.p.m. F during the period 1934 through 1948. The study was made in 1948. Monthly analyses by Elvove (*28*) in 1933 and 1934 averaged 3.9 p.p.m. F. In 1948, McClure found 3.3 p.p.m. F in a single sample of water. The water supply in Lubbock has been more constant in fluoride content. In 1933 and 1934, it averaged 4.4 p.p.m. F (*28*); in 1943, 3.8 p.p.m. F (*8*); and in 1948, 3.5 p.p.m. F. The epidemiological data on the distribution of mottled enamel in these two communities reported by Dean (7) are in agreement with these analyses (see table 1).

Water supplies in the Texas Panhandle generally contain from 2.0 to 5.0 p.p.m. F (*7*). Anyone living in Amarillo or Lubbock or in their wide environs would be exposed to fluoride drinking water.

Cumberland, in western Maryland, was selected as the control city. Its population is 37,000, and its water supply is

TABLE 1.—*Percentages of school children in Amarillo and Lubbock, Tex., affected by mottled enamel*[1]

Community	Signs absent		White opaque spots		Brown stains and pitting	
	Normal	Questionable	Very mild	Mild	Moderate	Severe
Amarillo, Tex.	3. 1	6. 6	15. 2	28. 0	33. 9	13. 2
Lubbock, Tex.	1. 1	1. 1	12. 2	21. 7	46. 0	17. 9

[1] Data from reference 7.

obtained by impounding surface water flowing in a nearby mountain stream. At the time of the study, the water contained 0.12 p.p.m. F. This low level of waterborne fluoride produces no mottled enamel in the Cumberland population.

STUDY PROCEDURES

Children selected for radiography had histories of continuous residence in the communities and continuous consumption of the local drinking water. This information was obtained by means of a questionnaire completed and signed by parents. No child with a history of absence from his community for more than 6 months at one time was included in the survey. Children aged 7 through 14 years were selected because they were accessible in the public schools and because this age span covers a period of rapid calcification and growth of the bones of the hand and wrist.

Mottled Enamel Diagnosis

While the children of Amarillo and Lubbock were at hand for their X-ray photographs, their teeth were inspected for mottled enamel. A record was made of the degree of mottling observed, using a simple comparative score of none, mild, moderate, or severe. The presence of moderate or severe mottled enamel is evidence of continuous exposure to fluoride drinking water during the period of tooth formation. Mild mottling or the absence of mottling may be attributed to reduced susceptibility to fluorine or to a reduced consumption of the local water supply during formative tooth life.

Radiographs

An X-ray picture showing the right hand and wrist of each child in the study was made in the school using portable equipment and a standard technique (*19*). This radiograph was produced on 5-inch x 7-inch no-screen film in a cardboard holder with 10 milliampere-seconds exposure, 70 peak kilovolts output, and 32-inch target-film distance. It was identified by a serial number applied with lead numerals on exposure, corresponding to the number assigned to the questionnaire returned by the child's parent. For densitometric purposes, an ivory step tablet was included in each exposure. Most of the films were developed in the field, usually on the day they were exposed. Radiographs of 2,050 children—965 boys and 1,085 girls—were used in the study. Distribution of the children by age and community of continuous residence is given in table 2.

Ossification Index

The films were used to establish a quantitative index of skeletal development for each child, employing the technique of Carter (*24*). According to this method, the areas of the shadows of the eight carpal bones and of the radial and ulnar epiphyses are measured with a polar planimeter. Their sum, the total ossified area, is divided by the area of a quadrilateral anatomically described for each film. The quotient is the ossification ratio. This ratio is an index of carpal ossification of the child, subject of the radiograph, corrected for his size, and therefore comparable to like indexes derived for other children. It reflects the status of development of the skeleton as a whole and may be correlated with chronological age in normally growing children.

In a test of reliability, the ossification ratio was determined from each of 50 radiographs by McCauley. Later, without the knowledge of indexes previously obtained, a laboratory assistant repeated the planimetry and calculation of the ossification ratio from the same films. The second determinations were correlated with the first by coefficient 0.975. This index was similarly reliable in the hands of Flory (*19*).

Skeletal Age Rating

The same films were used to obtain a skeletal age rating for each child. This rating is a qualitative evaluation of osseous development arrived at by comparison of the child's radiograph with a series of radiographs of the hand representing standards of maturation at varying levels of chronological age. Individual films, and therefore children, were assigned a developmental age.

For purposes of this study, skeletal age was determined by observation of (*a*) the contours of the metacarpal and phalangeal epiphyses, (*b*) the progress of their union with the diaphyses, (*c*) the presence or absence of each of the eight carpal bones and of the distal epiphyses of the radius and ulna, and (*d*) their morphology and stage of diaphyseal union. The assigned rating was that of the standard radiograph in which the above determinants of maturity most nearly approached those observed in the film being rated. This method, described originally by Todd (*20*) and more recently by Greulich and Pyle (*21*), supplements the quantitative method of Carter and offers a convenient control.

The standard radiographs used in the present study were obtained from the Brush Foundation. They were selected by Todd and his coworkers from radiographs of Cleveland children included in their extensive investigations at the Western Reserve University. Two series were employed, one for boys and another for girls. In the ages 7 through 14, each series contained a standard of osseous

TABLE 2.—*Distribution of children studied, by age, sex, and community of continuous residence*

Age last birthday (years)	Amarillo, Tex.		Lubbock, Tex.		Cumberland, Md.	
	Boys	Girls	Boys	Girls	Boys	Girls
7	16	27	32	38	41	40
8	44	56	46	44	48	46
9	45	64	47	48	48	60
10	29	46	37	51	54	63
11	37	27	42	30	62	44
12	39	34	43	52	47	51
13	36	39	47	56	32	47
14	21	31	36	41	36	50
All ages	267	324	330	360	368	401

development in the hand at 6-month intervals. Similar standards are available in the publications of Flory (*19*), Todd (*20*) and Greulich (*21*).

All assessments of skeletal age were made by McCauley. However, to test the reliability of the method, 67 radiographs each received an additional independent rating by one or another of three fellow laboratory workers. The duplicate ratings were correlated with the original by the coefficient 0.906. Flory (*19*) reported skeletal age ratings 87 to 97 percent reliable, depending on the training of the worker and the care with which the determinations are made. Todd (*20*) found that assessment of skeletal maturity from the hand was accurate to 6 months and associated with a reliability coefficient of 0.9.

AGE GROUPINGS

To provide the maximum numerical base for determining mean age-specific ossifi-

TABLE 3.—*Mean ossification ratios, and their standard errors, of carpal bones of normal children and of children exposed and not exposed to high-fluoride waters*

Average chronological age (months)	Fluoride water (3.5–4.5 p.p.m. F) Lubbock, Tex.		Fluoride water (3.3–6.2 p.p.m. F) Amarillo, Tex.		Mottled enamel, moderate or severe, Lubbock and Amarillo, Tex.	
	Number of children	Mean ossification ratio	Number of children	Mean ossification ratio	Number of children	Mean ossification ratio
Boys						
96	69	46. 62±1. 08	42	48. 94±1. 71	53	49. 29±1. 39
108	93	53. 24±1. 07	89	55. 87±1. 19	116	55. 11±0. 99
120	79	60. 34±1. 11	62	61. 73±1. 08	111	61. 05±0. 89
132	79	68. 73±1. 18	66	70. 69±1. 52	110	69. 03±0. 95
144	85	77. 54±1. 38	75	79. 51±1. 31	122	77. 78±1. 07
156	90	85. 22±1. 42	69	89. 13±1. 40	118	86. 68±1. 17
168	70	94. 42±1. 30	38	99. 29±1. 60	87	94. 87±1. 23
Girls						
96	75	58. 55±1. 21	61	61. 24±1. 28	67	60. 58±1. 32
108	90	66. 65±1. 26	120	71. 16±1. 12	130	69. 41±1. 05
120	99	76. 27±1. 17	94	82. 00±1. 28	133	79. 02±1. 03
132	63	86. 29±1. 42	59	92. 59±1. 48	92	89. 42±1. 31
144	78	95. 22±1. 02	57	99. 85±1. 05	90	97. 36±0. 90
156	108	101. 91±0. 80	72	104. 27±0. 80	135	103. 25±0. 65
168	86	107. 04±0. 61	56	109. 13±0. 92	99	107. 95±0. 59

Average chronological age (months)	Mottled enamel, zero or mild, Lubbock and Amarillo, Tex.		Nonfluoride water, no mottled enamel, Cumberland, Md.		University of Chicago normal children	
	Number of children	Mean ossification ratio	Number of children	Mean ossification ratio	Number of children	Mean ossification ratio
Boys						
96	33	45. 71±2. 02	81	47. 79±1. 13	100	52. 46±0. 90
108	54	52. 41±1. 54	96	54. 99±1. 19	100	60. 12±0. 86
120	25	61. 24±2. 02	99	61. 79±1. 14	100	68. 89±0. 96
132	27	70. 50±2. 47	116	69. 38±1. 09	100	75. 82±0. 99
144	33	80. 86±2. 31	99	80. 93±1. 20	100	84. 57±1. 01
156	33	91. 25±1. 80	67	89. 73±1. 61	100	95. 43±0. 87
168	23	99. 33±2. 23	67	98. 79±1. 31	100	101. 17±0. 77
Girls						
96	56	60. 41±1. 50	80	62. 17±1. 17	100	65. 34±0. 90
108	59	69. 10±1. 81	103	71. 27±1. 17	100	75. 73±0. 99
120	48	80. 55±1. 88	123	81. 89±1. 05	100	84. 58±0. 92
132	30	89. 30±1. 88	93	91. 76±1. 27	100	93. 48±0. 80
144	28	95. 96±1. 67	95	100. 66±0. 96	100	101. 30±0. 76
156	44	101. 72±1. 28	97	106. 03±0. 63	100	105. 27±0. 64
168	42	107. 62±1. 06	97	109. 71±0. 69	100	108. 34±0. 58

cation indexes and skeletal age ratings, the films of all the children in the study were separated by sex, then listed in the order of age at the time the radiographs were exposed. The radiographs were then divided into seven overlapping yearly age groups with average ages 96, 108, 120, 132, 144, 156, and 168 months, or 8 through 14 years. Each group consisted of children whose ages did not differ from the group average by more than 6 months. For instance, a group appearing in the tables of this report as having an average chronological age of 96 months (8 years) is composed of children 7 and 8 years old on their last birthday, consistent with the 96-month mean. Similarly, the group of average age 108 months (9 years) includes children 8 and 9 years old, and the group of average age 10 consists of 9- and 10-year olds.

RESULTS

Mean carpal ossification indexes and skeletal age ratings, together with their

TABLE 4.—*Mean skeletal age ratings, and their standard errors, of carpal bones of normal children and of children exposed and not exposed to high-fluoride waters*

Average chronological age (months)	Fluoride water (3.5–4.5 p.p.m. F) Lubbock, Tex.		Fluoride water (3.3–6.2 p.p.m. F) Amarillo, Tex.		Mottled enamel, moderate or severe, Lubbock and Amarillo, Tex.	
	Number of children	Mean skeletal age rating (months)	Number of children	Mean skeletal age rating (months)	Number of children	Mean skeletal age rating (months)
Boys						
96	69	85.35±1.37	42	88.29±2.09	53	89.04±1.62
108	93	96.94±1.11	89	99.20±1.75	116	99.21±1.46
120	79	111.00±1.87	62	109.94±1.76	111	110.30±1.50
132	79	126.72±1.74	66	125.09±2.14	110	125.62±1.53
144	85	140.29±1.48	75	138.20±1.51	122	138.84±1.20
156	90	150.73±1.64	69	151.00±1.70	118	150.61±1.46
168	70	163.71±1.72	38	165.79±2.52	87	163.34±1.72
Girls						
96	75	89.00±1.29	61	90.05±1.20	67	90.04±1.22
108	90	97.60±1.24	120	99.80±1.11	130	99.09±1.07
120	99	108.58±1.36	94	113.68±1.51	133	110.82±1.22
132	63	124.43±1.93	59	129.81±2.04	92	126.72±1.68
144	78	142.54±1.55	57	145.11±1.91	90	143.60±1.45
156	108	157.17±1.39	72	158.67±1.41	135	157.44±1.14
168	86	170.23±1.27	56	175.07±2.04	99	171.57±1.30

Average chronological age (months)	Mottled enamel, zero or mild, Lubbock and Amarillo, Tex.		Nonfluoride water, no mottled enamel, Cumberland, Md.		University of Chicago normal children	
	Number of children	Mean skeletal age rating (months)	Number of children	Mean skeletal age rating (months)	Number of children	Mean skeletal age rating (months)
Boys						
96	33	81.91±2.45	81	87.22±1.54	50	96.7±0.88
108	54	94.78±2.15	96	99.19±1.64	50	105.2±1.23
120	25	106.56±3.11	99	111.42±1.71	50	117.5±1.13
132	27	126.33±3.40	116	123.76±1.67	50	128.4±1.17
144	33	140.64±2.51	99	141.48±1.37	50	140.8±0.84
156	33	153.55±1.88	67	152.55±1.96	50	152.8±0.94
168	23	167.35±3.04	67	164.46±1.67	50	167.4±1.25
Girls						
96	56	89.57±1.39	80	90.98±1.12	50	95.8±1.26
108	59	98.28±1.64	103	101.85±1.10	50	108.2±0.99
120	48	112.50±1.97	123	113.15±1.19	50	119.9±0.94
132	30	126.60±2.75	93	127.71±1.79	50	133.0±1.07
144	28	144.85±2.39	95	144.60±1.55	50	145.8±1.28
156	44	158.32±2.11	97	159.00±1.18	50	158.0±1.30
168	42	173.00±2.27	97	170.38±1.42	50	170.5±1.00

standard errors, for boys and girls in the age groups described above are given in tables 3 and 4. Excluding standard errors, these data are presented graphically in figures 1 through 4. Weighted mean ossification indexes and skeletal age ratings for the groups combined for all ages and for mottled enamel experience are presented in figure 5. These tables and graphs also include data obtained by Flory (*19*) for University of Chicago normal children which are suitable for comparison with the data from this study. Uniformity of the data for individual children and among the groups is apparent in the standard errors of means of the data for each age group. Relatively large numbers of individuals are included in each age group. In general, the results appear satisfactory for reasonably sound conclusions.

The ossification ratios for boys of Amarillo, Lubbock, and Cumberland are uniform throughout, with the exception of those for Lubbock boys aged 13 and 14 years, which indicate a somewhat retarded rate of calcification (table 3, fig. 1). The data for boys in all three cities, however, indicate a bone development less rapid than for the University of Chicago normal children. The data for girls indicate a consistently lower rate of calcification for Lubbock girls than for any other group of girls. The other groups of girls, including the University of Chicago normal children, are quite uniform throughout, as shown in figure 1. The data showing ossification ratios of children classified according to the mottled enamel diagnosis indicate uniformity for all ages and for boys and girls in all groups (table 3, fig. 2).

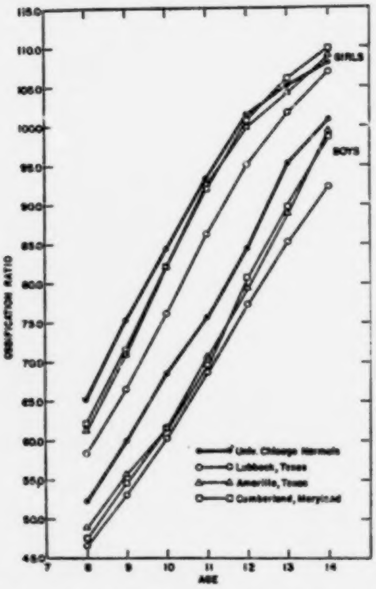

Figure 1.—*Mean ossification ratios of carpal bones.*

In figure 5, the mean carpal ossification ratios for boys and girls of all ages are compared graphically. The Lubbock boys and girls had the lowest values, and the value for Lubbock boys corresponds to that for boys of both Amarillo and Lubbock who showed little or no mottled enamel. The girls in Lubbock and Amarillo who had moderate or severe mottled enamel compared favorably with both the Cumberland and the University of Chicago girls and with the girls in Lubbock and Amarillo diagnosed as having only mild or no mottled enamel.

SKELETAL AGE RATINGS

For ages 8 through 11 years, the skeletal age ratings for the children in Lubbock, Amarillo, and Cumberland differ from the ratings for the University of Chicago children (table 4, fig. 3). However, at ages 12, 13, and 14 years, all groups agreed remarkably well. When the children were classified according to mottled enamel diagnosis, all groups demonstrated uniformity in skeletal age ratings (table 4, fig. 4).

As shown in figure 5, the data for mean skeletal age ratings suggest some advanced development for Lubbock girls and perhaps for both boys and girls of Amarillo and Lubbock who had moderate or severe mottled enamel. This finding was not apparent, however, when the Amarillo boys and girls were considered as a group regardless of the mottled enamel diagnosis.

SEX DIFFERENCES

The repeated observations of advanced skeletal maturity in girls, as compared with boys, from birth through the growing years is confirmed in the findings in this study. Sex differences in skeletal development are particularly evident in comparisons of the ossification ratios (tables 3 and 4, figs. 1, 2, and 5). They are suppressed in comparisons of skeletal age ratings because of the use of separate standards for boys and girls (figs. 3, 4, and 5). Between ages 7 and 12 years, average osseous development in the girls apparently proceeded approximately at the same rate as in boys, as shown by the slope of the curves, but was advanced about 2 years over that of the boys. This lead was reduced in the next 2 years as the girls approached maturity and the boys continued to grow at the same rate as before.

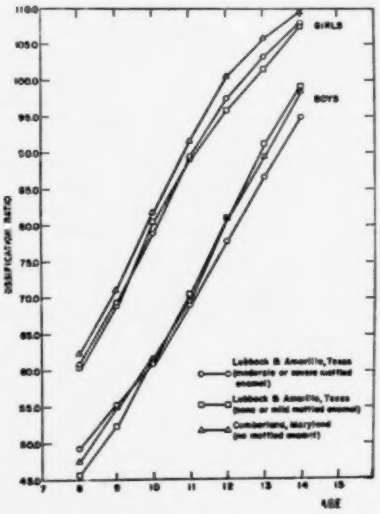

Figure 2.—*Mean ossification ratios of carpal bones.*

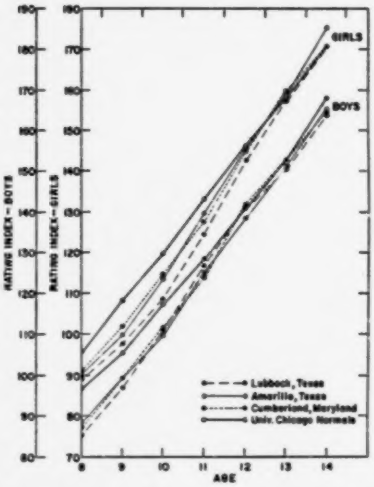

Figure 3.—*Skeletal age rating index as determined by carpal bone calification.*

DISCUSSION

Continuous exposure to relatively high levels of waterborne fluoride apparently failed to influence the calcification of the carpal bones of children aged 7 through 14 years. This result appears evident in the high order of agreement in the data obtained on three location groups of children, with and without exposure to waterborne fluoride.

Minor differences occurred in the weighted mean carpal ossification ratios and in the mean skeletal age ratings for the University of Chicago, Amarillo, Lubbock, and Cumberland children, but these differences appear not to be associated with the exposure to high-fluoride water received by the Amarillo and Lubbock children. The data suggest some tendency toward a uniformly lower osseous development among Lubbock children, particularly girls, but not among Amarillo girls or boys. However, since children in both communities were exposed to fluoride waters, this suggested difference may be related to other factors.

It has been reported that the progress of ossification may be influenced by environmental conditions (*29, 30*), extremes in climate (*31*), systemic disorders (*30, 32*), and endocrine functions (*32*). Lubbock and Amarillo are within 150 miles of each other, but they differ somewhat in environmental surroundings. Lubbock is a relatively new community, the seat of a large technologic college, and the center of an agricultural area. Amarillo is a much older town and is surrounded by cattle ranges. The populations in the two communities may therefore differ somewhat in their hereditary and ethnic background and in general day-to-day activities. The effects of environmental variables on bone development among the children of these towns, however, are purely speculative.

As previously mentioned, the most pronounced nondental effect of excessive quantities of fluorine is an advanced calcification in both skeletal and ligamentous tissues (*1–4*). Experimental studies with animals have also given evidence that a high-fluorine intake is identified with skeletal hypercalcification (*33–36*). Results in these latter studies are based generally on analytical data.

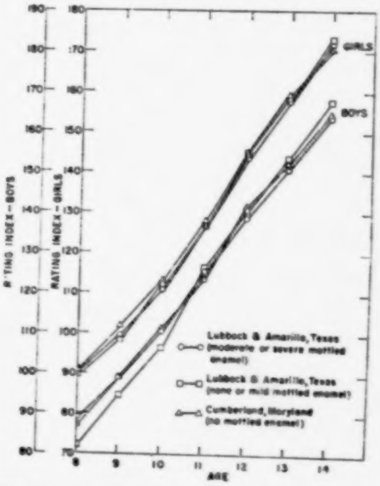

FIGURE 4.—*Skeletal age rating index as determined by carpal bone calification.*

Inconsistencies in the data are attributed mainly to variations in calcium intake as well as to the extent and duration of the fluoride exposure. In general, relatively excessive quantities of fluoride must be ingested by experimental animals to produce calcification changes, as shown by ash, calcium, or phosphorus analyses. For example, in a recent report (*36*), as much as 50 p.p.m. F in the drinking water of growing experimental rats caused a more than fivefold increase in the fluorine content of mandibles and femurs without affecting the amount of ash, calcium, or phosphorus or the calcium: phosphorus ratio of the ash. More recent attempts to assess the relation of fluorine to calcium metabolism of skeletal tissues have made use of Ca^{45} (*37, 38*). These studies have failed to produce evidence of any pronounced effect of relatively high levels of fluorine on the calcium metabolism of skeletal tissues.

A survey of the previous experiences with fluorine, both clinically and experimentally, in respect to skeletal tissue development, therefore, gives every reason to expect that if any effects were to be found in the present study of the development of the carpal bone in children, they would be in the nature of hypercalcification and advanced skeletal maturity and bone development. The calcification data accumulated in this study do not indicate the occurrence of this characteristic skeletal effect of fluorine among the children drinking the fluoride waters of Lubbock and Amarillo. Apparently, approximate levels of 3.5 to 5.0 p.p.m. F in the drinking water are not sufficient to affect calcification in skeletal tissues which are developing at ages 7 through 14 years. The presence of 3.5 to 5.0 p.p.m. F in the drinking water also has not altered the long-established sex differences in the rate of development.

Previously in this report, evidence was cited which showed that the development of bones of the hand and wrist parallels that of the rest of the skeleton. On the basis of this evidence, it seems justified to suggest that skeletal development throughout the body was not affected by the exposure to high-fluoride water received by the Lubbock and Amarillo children. There may be some consistency in this finding and previous evidence that age, height, and weight relations and bone-fracture experience are not influenced by exposure to high-fluoride waters (*10*). A number of the persons included in the previous study lived in Amarillo and Lubbock or their immediate environs.

The results of this study apply to the continuous exposure to drinking water containing approximately 3.5 to 6.2 p.p.m. F, quantities considerably larger than the approximately 1.00 p.p.m. F added to drinking water for the partial control of dental caries. There is every justification for concluding from these results, therefore, that this added 1.00 p.p.m. will not be detrimental to the skeletal development of children as shown by the progress of calcification in the carpal bones.

SUMMARY

Exposure to fluoride in drinking water was studied for evidence of detrimental effects on skeletal calcification and bone development in children. Three groups of children aged 7 through 14 years, living in Lubbock and Amarillo, Tex., and Cumberland, Md., were selected on the basis of continuous exposure to their communal drinking waters, which contained fluoride in the amounts of 3.5 to 4.5 p.p.m. F, 3.3 to 6.2 p.p.m. F, and 0.1 p.p.m. F, respectively. Radiographs were taken of the right hand and wrist of 2,050 children. From these X-rays, the skeletal age was assessed and a quantitative index of ossification was determined.

No evidence, available by radiographs, was obtained which would indicate that there was any adverse effect on the carpal bones or on their growth and development as a consequence of the continuous use of drinking water containing approximately 3.5 to 6.2 p.p.m. F. These results confirm the safety of maintaining the fluoride level of public water supplies at about 1.00 p.p.m. F, by controlled fluoridation, for the reduction of tooth decay.

ACKNOWLEDGMENTS

Acknowledgment is made to the staffs of the public schools in Amarillo and Lubbock, Tex., and Cumberland, Md., for their cooperation and assistance in taking the X-ray pictures; J. W. Wyatt, city chemist of Amarillo, for providing data on fluorine content of the water; and Saint Anthony's Hospital in Amarillo, Doctors Hospital in Lubbock, and Allegheny Hospital in Cumberland, for use of their darkrooms to develop the films.

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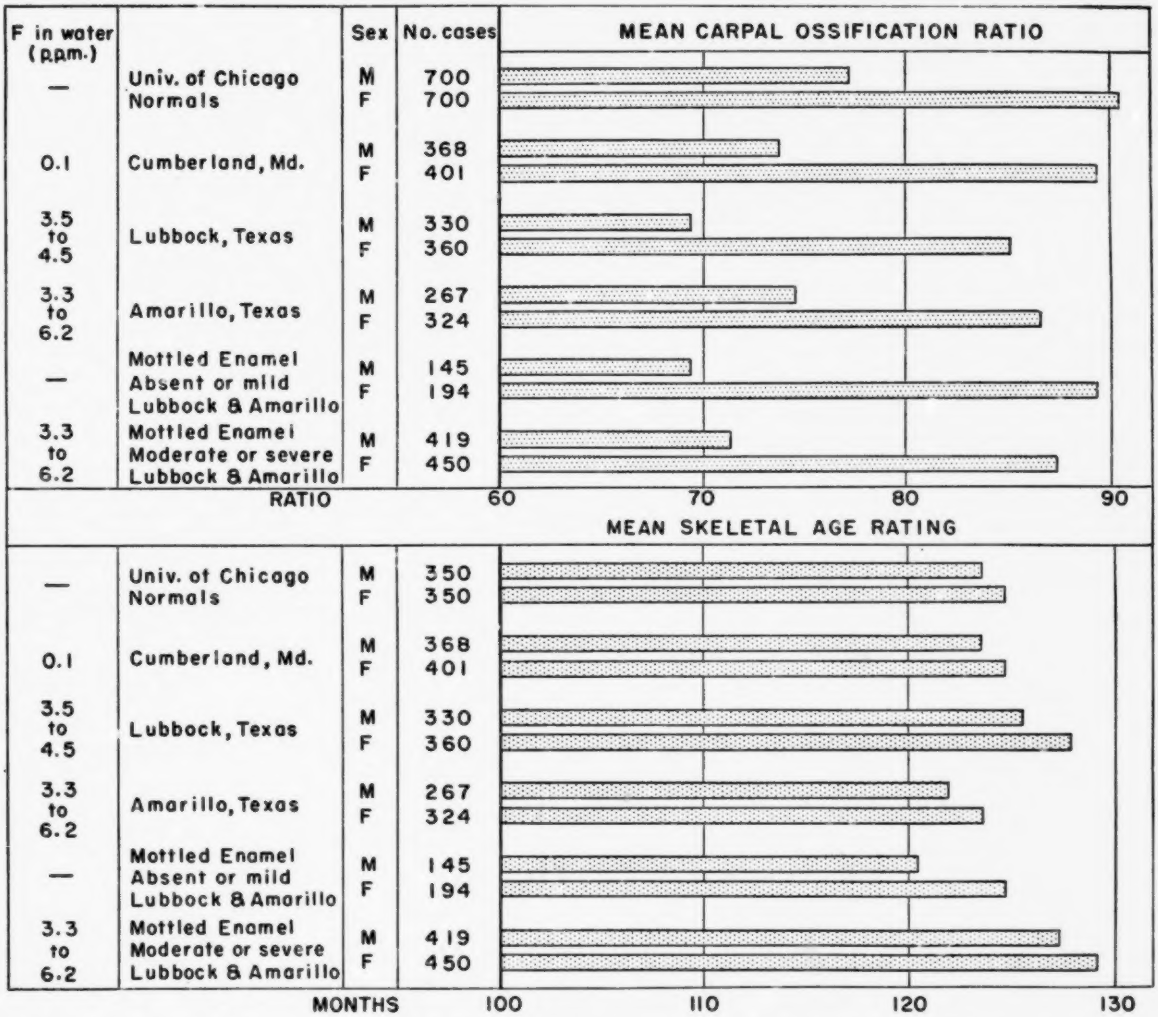

FIGURE 5.—*Mean carpal ossification ratios and skeletal age ratings for groups of children with and without exposure to high-fluoride waters.*

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MEDICAL ASPECTS OF EXCESSIVE FLUORIDE IN A WATER SUPPLY
A TEN-YEAR STUDY

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This is a report of a study of a group of people living in Bartlett and Cameron, Tex. It is one of several related studies designed to provide information on the effects of excessive amounts of fluoride occurring naturally in a water supply.

Previous investigations (*1*, *2*, *3*) indicate a need for additional information on the effects of prolonged fluoride exposure. Moreover, other studies have related, in a limited number of people, physiological reactions to the presence of excess fluoride, but have not defined the prevalence or incidence of these characteristics in the exposed group (*2*, *3*, *4*).

Bartlett, Tex., was selected as a study area because the town's water supply contained 8 p.p.m. F, one of the higher fluoride levels of public drinking water in the United States. A town with a water supply naturally containing excessive fluoride was selected on the assumption that any association between exposure and physiology might be manifest at a high concentration, particularly after prolonged ingestion. Cameron, Tex., was selected as the control study area. The Cameron water supply contains 0.4 p.p.m. F. Both towns are similar with respect to geography, racial composition, and principal occupation, which is agriculture. Each participant in the study was required to have been a resident of one of these towns for 15 consecutive years prior to 1943.

The medical history of the participants since birth was recorded at the start of the study, and their medical experience during the ten-year period 1943–1953 was carefully reviewed. This permitted a comparison of the individuals in each group with themselves, and of those in a high-fluoride group with those in a low-fluoride area.

The study has produced findings of two types—those that can be enumerated and analyzed by statistical techniques and those that are classified as clinical impressions. The latter are more difficult to evaluate, but they are important in the analysis of specific clinical problems.

This paper is concerned with the statistical analysis of the results of the study. Clinical impressions will be evaluated in subsequent papers, in which the medical, X-ray and dental findings will be presented in more detail.

CHARACTERISTICS OF STUDY AREAS

Bartlett and Cameron are geographically located on a triangle with Temple, Tex., 135 miles due south of Dallas. Bartlett is 20 miles south of Temple, and Cameron 38 miles southeast of Temple. The two towns are about 25 linear miles apart but are not connected by a direct road. In 1940 the populations of Bartlett and Cameron were 1,668 and 5,040; and in 1953, 1,706 and 5,272, respectively.

Prior to 1943, there were usually two practicing physicians and one dentist in Bartlett; ten physicians and six dentists in Cameron. During the 1953 survey, there was one practicing physician and one dentist in Bartlett, and eight physicians and four dentists in Cameron. Bartlett residents are also seen by physicians in nearby Temple and Taylor, and Cameron residents are served by the medical profession in Temple and Marlin.

Bartlett.—The municipal water supply of Bartlett, serving the entire town, comes from two deep wells of approximately the same depth. Well No. 1 was drilled to 2,005 feet in 1901; well No. 2, to 1,595 feet in 1936. By the latter year, both were plugged to 1,404 feet. Both wells are now pumping at the 240-foot level, each with a freeflowing capacity output of 220 gallons per minute.

Until March 11, 1952, the raw water was pumped into three storage tanks, from which it entered directly into the general distribution system (5). Repeated analyses of the Bartlett water supply have shown a fluoride level ranging from 7.6 to 8.2 p.p.m. F. Few sizable communities in the United States use water supplies with a fluoride concentration in excess of 6 p.p.m. F.

Table 1 shows the typical composition of the raw water supplies of Bartlett and Cameron during the period of study. On March 11, 1952, an experimental defluoridation unit was installed in Bartlett, and the table also shows the composition of the defluoridated water on the day the raw water was sampled.

Since the installation of the defluoridation unit, the fluoride content of the water has varied considerably. However, it has remained somewhat above the desired level of 1.0 p.p.m. F. "Optimal defluoridation" has not been achieved without interruption because of necessary changes in the experimental unit (5). By 1953 the Bartlett participants in the study had ingested fluorides at high levels for an average of 36.7 years. Any significant physiological manifestations of prolonged exposure would not be expected to have regressed materially in the 18 months of partial defluoridation.

Cameron.—Cameron has taken its raw water from the Little River since 1895. Sedimentation basins were installed in 1911 and floored in 1929. The water has been alum-treated since 1911, chlorinated since 1919, and carbon-treated since 1936. The present treatment consists of prechlorination, coagulation with alum and lime, sedimentation, and postchlorination. Occasionally, activated carbon is added for the control of taste and odor. The plant is not equipped with filters. In 1953 a pressure tank was added to the system. (See table 1 for water analyses.)

PROCEDURE

In 1943 a medical-dental team from the Public Health Service examined 116 participants in Bartlett and 121 in Cameron. At that time, the participants' ages ranged from 15 to 68 years in both towns. All were of the white race.

The 1943 group was selected by a survey covering approximately every household in Bartlett and every third household in Cameron. An adult member of

Table 1.—*Composition of water*[a]—*Bartlett and Cameron, 1943 and 1953*

Components	Bartlett			Cameron	
	Raw Water		Defluoridated	Raw Water	
	Jan. 1943	Dec. 1953	Dec. 1953	Jan. 1943	Dec. 195
Dissolved solids	1698.4	1708.8	1701.2	435.2	363.6
Loss on ignition	40.0	34.4	16.0	66.4	60.8
Fixed residue	1658.4	1674.4	1685.2	368.8	302.8
Silica (SiO_2)	15.2	16.0	17.6	6.4	5.6
Iron (Fe)	0.03	0.01	0.01	0.02	0.02
Aluminum (Al)	0.0	0.00	0.02	0.07	0.01
Calcium (Ca)	19.4	24.6	21.2	74.3	85.8
Magnesium (Mg)	11.5	11.4	11.4	20.6	8.4
Sodium and potassium (calculated as Na)	588.9	585.7	582.2	46.6	22.6
Carbonate (CO_3)	0.0	7.2	2.4	0.0	1.2
Bicarbonate (HCO_3)	485.6	458.7	451.4	258.6	168.0
Sulfate (SO_4)	482.2	471.9	493.6	65.8	93.0
Nitrate (NO_3)	2.2	0.22	1.33	4.4	4.2
Chloride (Cl)	319.0	325.9	323.5	63.0	30.4
Phosphate (PO_4)	0.0	0.0	0.0	0.0	0.0
Fluoride (F)	8.0	7.6	1.2	0.4	0.5

[a] The 1943 water analyses were made by Elias Elvove–the fluoride determinations, by means of the zirconium-alizarin reagent (6). The 1953 fluoride analyses were done by F. J. McClure (7). Both investigators are from the National Institutes of Health. Phosphates were determined by an adaptation of the colorimetric method of Benedict and Theis (8). Determinations other than fluoride and phosphate were made according to the *Standard Method of Water Analysis* of the American Public Health Association.

each household was interviewed and a "Household Roster" card was filled out. In addition, a "Residence and Water History" card was made for each household member. On the basis of the data then obtained, a random sample was selected from those who met the basic requirement, 15 years or more of continuous residence prior to 1943.

The 1953 investigation involved the location and study of the group 10 years later and a reexamination of as many participants as possible.

The 1943 and 1953 investigation procedures were essentially parallel. These included a medical history, physical and dental examinations, X-rays, and blood and urine studies. Both sets of data were recorded on punch cards to facilitate comparison. In the earlier study, the physical and dental examinations and the laboratory work were done locally in the respective towns, but the patients were later transported to the Scott and White Clinic in Temple, Tex., for X-rays.

The 1953 procedure differed slightly in that the majority of physical and laboratory examinations, as well as X-rays, were performed at the Scott and White Clinic by Public Health Service personnel. Blood and urine analyses were done by the Scott and White Clinic technicians, and the chief of the X-ray department supervised the taking of X-ray films and made the initial interpretations. The films were later evaluated independently by Theodore F. Hilbish, Chief of the Department of Diagnostic X-ray, Clinical Center, National Institutes of Health, and Merrill C. Sosman, Radiologist, Peter Bent Brigham Hospital, Boston, and radiology consultant to the National Institutes of Health.

The 1943 examining team consisted of J. W. Hawkins, M. B. Shimkin, and Chester Brown, physicians; F. A. Arnold, Jr., dentist; Christine Mason and Mary Harte, nurses; and Stoel Davis, technician.

The 1953 team consisted of N. C. Leone, physician; E. R. Zimmerman, dentist; Patricia Geiser, Public Health Nurse; Vona Harrell, F. D. Baty, and Elaine Pickett, assistants.

Approximately 71 percent of the 237 participants in the 1943 survey still resided in Bartlett and Cameron in 1953. Information on the deceased, another 8 percent, was obtained from the next of kin, and copies of the death certificates were obtained to establish cause of death. Because of the unusual success in accounting for 79 percent of the participants locally, it was deemed advisable to follow up the remaining 21 percent of the participants who had moved from the study areas. All of the 1943 participants, living and deceased, were accounted for in the 1953 study.

Table 2.—*Distribution of participants by locality, age, and sex, based on 1943 age*

Age Group	Bartlett			Cameron		
	Male	Female	Total	Male	Female	Total
15–24	5	16	21	11	7	18
25–29	—	—	—	—	5	5
30–34	2	4	6	1	6	7
35–39	5	11	16	6	12	18
40–44	1	7	8	6	11	17
45–49	7	6	13	11	15	26
50–54	7	15	22	4	8	12
55–59	3	11	14	4	9	13
60–64	7	3	10	2	1	3
65–69	2	3	5	1	1	2
70+	—	1	1	—	—	—
Total	39	77	116	46	75	121

Table 3.—*Distribution of participants by locality, age, and sex, based on 1953 age*

Age Group	Examined, 1943			Examined, 1953			Not Examined	Deceased		
	Male	Female	Total	Male	Female	Total		Male	Female	Total
					Bartlett					
25–29	5	12	17	5	9	14	3	—	—	—
30–34	—	4	4	—	2	2	3	—	—	—
35–39	—	—	—	—	—	—	—	—	—	—
40–44	2	3	5	2	3	5	—	—	—	—
45–49	4	11	15	4	10	14	—	—	—	—
50–54	2	6	8	2	6	8	—	—	—	—
55–59	6	8	14	3	8	11	—	3	—	3
60–64	10	13	23	8	13	21	—	1	1	2
65–69	2	11	13	1	9	10	—	1	2	3
70+	8	9	17	6	5	11	—	3	3	6
Total	39	77	116	31	65	96	6	8	6	14
					Cameron					
25–29	11	6	17	9	6	15	2	—	—	—
30–34	—	1	1	—	1	1	—	—	—	—
35–39	—	5	5	—	4	4	1	—	—	—
40–44	1	4	5	1	4	5	—	—	—	—
45–49	6	13	19	6	13	19	—	—	—	—
50–54	6	11	17	5	11	16	—	1	—	1
55–59	11	13	24	11	12	23	1	—	—	—
60–64	4	10	14	4	10	14	—	—	—	—
65–69	4	10	14	2	9	11	—	2	—	2
70+	3	2	5	2	3	5	—	1	—	1
Total	46	75	121	40	73	113	4	4	—	4

A total of 47 participants had moved from the two towns by 1953. Of these, 37 were examined and the data were obtained by the same procedures as used for the persons who remained. Forty-three of the "removed" participants still lived in Texas. Two had moved to California, one to Oklahoma, and one to New York City.

The 10 living participants who were

not examined were interviewed by personal contact, telephone, or mail, and a 10-year medical and residence history was obtained. None reported acute, chronic, serious, or debilitating illnesses.

Physical Examination.—In 1943 and 1953 a medical and residence history was obtained in each case. At the conclusion of the examinations, a summary of the findings was prepared for the family physician.

The laboratory blood studies in 1943 and 1953 included a determination of hemoglobin, red and white blood counts, a differential white count, and a serologic test for syphilis. Urine determinations included specific gravity, qualitative albumin and sugar, and microscopic examination.

Table 4.—*Distribution of removed participants, 1943–1953*

Age Group	Examined			Not Examined[a]			Total
	Male	Female	Total	Male	Female	Total	
				Bartlett			
25–29	2	7	9	—	3	3	12
30–34	—	1	1	—	3	3	4
35–39	—	—	—	—	—	—	—
40–44	1	—	1	—	—	—	1
45–49	—	—	—	—	—	—	—
50–54	—	1	1	—	—	—	1
55–59	—	—	—	—	—	—	—
60–64	1	1	2	—	—	—	2
65–69	—	2	2	—	—	—	2
70+	—	—	—	—	—	—	—
Total	4	12	16	—	6	6	22
				Cameron			
25–29	8	3	11	2	—	2	13
30–34	—	1	1	—	—	—	1
35–39	—	2	2	—	1	1	3
40–44	—	—	—	—	—	—	—
45–49	1	1	2	—	—	—	2
50–54	—	2	2	—	—	—	2
55–59	1	1	2	—	1	1	3
60–64	—	1	1	—	—	—	1
65–69	—	—	—	—	—	—	—
70+	—	—	—	—	—	—	—
Total	10	11	21	2	2	4	25

[a] Medical history in 1953, but no physical, laboratory, or x-ray examination.

Table 5.—*Percentage distribution of participants by age groups, based on 1953 age*

Age Group	Bartlett		Cameron	
	1943	1953	1943	1953
25–29	14.7%	14.6%	14.0%	13.3%
30–34	3.4	2.1	0.8	0.9
35–39	—	—	4.1	3.5
40–44	4.3	5.2	4.1	4.4
45–49	12.9	14.6	15.8	16.8
50–54	6.9	8.3	14.0	14.2
55–59	12.1	11.5	19.9	20.4
60–64	19.8	21.8	11.6	12.4
65–69	11.2	10.4	11.6	9.7
70+	14.7	11.5	4.1	4.4
Total	100.0	100.0	100.0	100.0

In 1953, additional procedures were included. Rectal and pelvic examinations of most of the females and prostate examinations in males were routine. Hematocrit, sedimentation rate, and blood calcium were determined; and when indicated, further studies were made, such as acid and alkaline phosphatase, blood sugar, and special hematology. Consultant service was used when indicated.

The removed participants were given essentially the same examination by the same Public Health Service physician.

X-Ray Examination.—The X-rays taken at Scott and White Clinic were made on a 500-ma radiographic unit with rotating anode tube. Emphasis was placed on bone detail. Anteroposterior views of the dorsal spine, the lumbar spine, and the pelvis with the proximal third of the femur were taken for each patient. When a question of unusual findings arose, a radiographic bone survey was made, consisting of the following views: lateral skull, cervical spine, left upper arm, forearm and hand, and right femur, lower leg and foot.

The removed participants were similarly X-rayed at the nearest available facility. All X-rays, regardless of where taken, were evaluated by the same three roentgenologists.

Oral Examination.—All physical examinations included an oral examination by the Public Health Service physician. In addition, the majority of participants seen at Scott and White Clinic in 1953 received a complete dental examination, including X-rays. Estimations were made on the degree and prevalence of dental fluorosis, caries, gingivitis, and alveolar bone loss. Dental X-rays were taken on all participants seen by the dentist, including the edentulous, and the films were evaluated independently by four dentists. The presence or absence of dental fluorosis in the removed participants was determined by the examining physician.

EVALUATION

Age and Sex Distribution of Participants.—Table 2 gives the age-sex distribution of the original 1943 participants. Table 3 gives the age-sex distribution of the participants in the 1943 and 1953 surveys and the deceased during the 10-year period. In this and subsequent tables, the age groupings are on the basis of the participants' ages in 1953. Table 4 gives the age-sex composition of the group that moved from the study area in the 10-year interval, and table 5 shows the percentage distribution of all examined participants by age group.

Table 3 shows that in Bartlett and Cameron, the ratio of male to female participants in both 1943 and 1953 is approximately 1 to 2. The male-to-female ratio for the entire populations in the towns is 0.9 to 1 in Bartlett and 1.1 to 1 in Cameron (*9, 10*).

The age distribution of the participants does not exactly parallel that of the populations. In 1943, 57.8 percent of the Bartlett participants were over 55 years of age (table 5), whereas persons 55 and over constituted about 50 percent of the total adult population. The comparable figures for Cameron are 47.2 percent and approximately 58 percent. This imbalance was due to the basic requirement of 15 years' continuous residence and the absence of males of military age.

The sex ratios of the participants were approximately the same in the two study areas, and the age distributions were similar though not exactly comparable, since in Bartlett they were predominantly older. The imbalance is particularly evident in the age group 70 and over. As a result the difference between the age-corrected mortality rates is not significant by statistical analysis. (See table 3 for age-sex distribution of the deceased.)

Removed Participants.—In table 4 the removed participants are subdivided into

TABLE 7.—*Incidence of abnormal clinical findings, 1943–1953 (removed participants)*

Characteristic Studied	Bartlett		Cameron	
	Number at Risk[a]	Number Abnormal	Number at Risk[a]	Number Abnormal
Arthritic change	16	0	21	2
Blood pressure				
Sys. 151 mm/Hg and over	16	2	19	1
Dias. 100 mm/Hg and over	16	0	21	0
Pulse pressure 75 mm/Hg and over	16	2	19	0
Bone changes[b]				
Density	15	0	20	1[c]
Coarse trabeculation	15	0	20	0
Hypertrophic	15	2	20	0
Spurs	15	0	20	0
Osteoporosis	15	0	20	0
Bone, increased density (new cases)	15	0	20	0
Cataract and/or lens opacity	16	2	21	1
Thyroid	16	0	19	4
Cardiovascular (except uncomplicated hypertension)	16	4	21	2
Hearing (decreased acuity)	16	0	21	1
Tumor and/or cysts	16	3	21	5
Fractures	22	2	25	2
Urinary tract calculi	16	0	21	2
Gall stones	15	0	20	0

[a] At risk for that part of the ten-year interval in which the participant resided in the study area.

[b] Bone changes determined by simultaneous reading of identical views of x-rays taken in 1943 and repeated in 1953.

[c] Cameron: 1 decreased density.

TABLE 6.—*Incidence of abnormal clinical findings, 1943–1953 (participants residing in study area for the 10-year period)*

Characteristic Studied	Bartlett			Cameron			Significant Difference ($P = 0.05$)
	Number at Risk	Number Abnormal	Rate, %	Number at Risk	Number Abnormal	Rate, %	
Arthritic change	80	11	13.8	89	13	14.6	No
Blood pressure							
Sys. 151 mm/Hg and over	58	18	31.0	81	20	24.7	No
Dias. 100 mm/Hg and over	73	11	15.1	83	11	13.3	No
Pulse pressure 75 mm/Hg and over	70	9	12.9	89	16	18.0	No
Bone changes[a]							
Density[b]	74	7	9.5	81	2	2.5	No
Coarse trabeculation	74	4	5.4	81	2	2.5	No
Hypertrophic	74	8	10.8	81	6	7.4	No
Spurs	74	1	1.4	81	4	4.9	No
Osteoporosis	74	5	6.8	81	10	12.3	No
Bone, increased density (new cases)	66	1	1.5	79	—	—	—
Cataract and/or lens opacity	79	8	10.1	85	12	14.1	No
Thyroid	74	3	4.1	82	6	7.3	No
Cardiovascular (except uncomplicated hypertension	80	10	12.5	92	22	23.9	Yes
Hearing (decreased acuity)	72	14	19.4	78	10	12.8	No
Tumor and/or cysts	80	12	15.0	92	10	10.9	No
Fractures	80	12	15.0	92	7	7.6	No
Urinary tract calculi	72	14	19.4	76	12	15.8	No
Gall stones	73	0	0.0	80	1	1.2	No

[a] Bone changes determined by simultaneous reading of identical views of x-rays taken in 1943 and repeated in 1953.

[b] Bartlett: 4 increased density, 3 decreased density. Cameron: 2 increased density.

Table 8.—*Incidence of abnormal clinical findings, 1943–1953 (resident and removed participants)*

Characteristic Studied	Bartlett			Cameron		
	Number at Risk	Number Abnormal	Rate, %	Number at Risk	Number Abnormal	Rate, %
Arthritic change	96	11	11.5	110	15	13.6
Blood pressure						
Systolic 151 mm/Hg and over	74	20	27.0	100	21	21.0
Diastolic 100 mg/Hg and over	89	11	12.4	104	11	10.6
Pulse pressure 75 mm/Hg and over	86	11	12.8	108	16	14.8
Bone changes[a]						
Density[b]	89	7	7.9	101	3	3.0
Coarse trabeculation	89	4	4.5	101	2	2.0
Hypertrophic	89	10	11.2	101	6	5.9
Spurs	89	1	1.1	101	4	4.0
Osteoporosis	89	5	5.6	101	10	9.9
Bone, increased density (new cases)	81	1	1.2	99	—	—
Cataract and/or lens opacity	95	10	10.5	106	13	12.3
Thyroid	90	3	3.3	101	10	9.9
Cardiovascular (except uncomplicated hypertension)	96	14	14.6	113	24	21.2
Hearing (decreased acuity)	88	14	15.9	99	11	11.1
Tumor and/or cysts	96	15	15.6	113	15	13.3
Fractures	102	14	13.7	117	9	7.7
Urinary tract calculi	88	14	15.9	97	14	14.4
Gall stones	88	0	0.0	100	1	1.0

[a] Bone changes determined by simultaneous reading of identical views of x-rays taken in 1943 and repeated in 1953.

[b] Bartlett: 4 increased density, 3 decreased density. Cameron: 2 increased density, 1 decreased density.

Table 9.—*Prevalence of abnormal laboratory findings, 1943 and 1953 (participants residing in study area for the 10-year period)*

Laboratory Determination	Year	Bartlett			Cameron			Significant Difference ($P = 0.05$)
		Number Examined	Number Abnormal	Rate, %	Number Examined	Number Abnormal	Rate, %	
Hemoglobin	1943	116	34	29.3	121	37	30.6	No
	1953	79	20	25.3	83	26	31.3	No
Hematocrit	1943	—	—	—	—	—	—	—
	1953	79	5	6.3	82	7	8.5	No
Red blood count	1943	116	25	21.6	121	24	19.8	No
	1953	80	6	7.5	85	2	2.4	No
White blood count	1943	116	17	14.7	121	5	4.1	Yes
	1953	78	11	14.1	82	7	8.5	No
Differential count								
Neutrophiles	1943	71	15	21.1	71	6	8.5	Yes
	1953	78	23	29.5	82	13	15.9	Yes
Lymphocytes	1943	71	2	2.8	71	1	1.4	No
	1953	78	35	44.9	82	36	43.9	No
Eosinophiles	1943	71	0	0.0	71	0	0.0	No
	1953	78	6	7.7	82	14	17.1	No
Sedimentation rate	1943	—	—	—	—	—	—	—
	1953	79	31	39.2	83	22	26.5	No
Blood calcium	1943	—	—	—	—	—	—	—
	1953	79	9	11.4	66	7	10.6	No
Serology (S.T.S.)	1943	71	2	2.8	71	3	4.2	No
	1953	84	2	2.4	95	2	2.1	No
Urine albumin	1943	115	3	2.6	121	10	8.3	Yes
	1953	77	5	6.5	85	12	14.1	No
Urine glucose	1943	115	2	1.7	121	4	3.3	No
	1953	77	0	0.0	85	1	1.2	No

those who were examined (including X-ray and laboratory studies) and those who were not. The number of persons that left each town was almost equal—22 from Bartlett and 25 from Cameron, predominantly of the younger age groups.

The average duration of continuous residence of all living participants examined was 36.7 years in Bartlett and 38.2 years in Cameron, and the corresponding averages for those who moved were 22.4 in Bartlett and 21.3 in Cameron.

Results.—Table 6 shows the 10-year incidence rates for selected clinical findings of those who were at risk for the entire 10-year period. Table 7 shows the 10-year incidence figures for the same characteristics studied in the removed participants. Table 8 is a composite of all those examined. Similarly, tables 9 and 10 show the prevalence data for laboratory and other observations, and table 11 is a composite of tables 9 and 10. In the subsequent evaluation of data, the differences between the findings for the two towns are subjected to statistical analyses in order to test the hypothesis that Bartlett did not differ from Cameron with respect to the characteristics studied. Each characteristic studied, laboratory determination, and dental finding was tested statistically for significant differences between towns. Approximately 40 tests were made. It was therefore expected that at least three characteristics would be judged significant as a consequence of the statistical procedure (*11*). It was necessary to use this statistical procedure because a main objective of the study was to determine which, if any, of the characteristics were important.

The number of persons "at risk," as the term is used in the various tables, is the number of individuals who had a chance of acquiring the "characteristic studied" during the 10-year interval. A participant was considered at risk only with respect to characteristics for which he was examined both in 1943 and 1953. The variation in the number at risk is largely due to the unavoidable omission of parts of examinations.

No attempt has been made to reconcile the differing concepts of "normal" with respect to the characteristics studied. Objective criteria were chosen to designate the "abnormals" and apply equally to both towns. The purpose of these criteria was to form a dichotomy of the participants with respect to each characteristic, to permit statistical evaluation. Appended is a glossary of ranges, standards, and definitions used.

Tables 6 and 9 show that significant differences were found between the two study areas in the incidence rates for cardiovascular abnormalities and the prevalence rates for white blood count, neutrophiles, and urine albumin.

As was expected, dental fluorosis was observed in Bartlett in all of the participants born and in continuous residence in Bartlett during the tooth formative period. In Cameron, the single individual with dental fluorosis had a history of early fluoride exposure. No new cases appeared in the participants of either area in the 10-year period.

Neither the statistically significant differences nor those that are not significant predominate in either group, except in the case of dental fluorosis. The other significant differences were just above the significance level. These differences were no greater than would be expected in a like study of any two areas similar to Bartlett and Cameron, with or without an excess of fluoride (F) in either water supply.

CONCLUSIONS

The incidence of specific systemic abnormalities and the prevalence of abnormal laboratory findings showed no tendency toward higher or lower rates for either town, except that the number of cases of dental fluorosis was significantly higher in Bartlett than in Cameron and the difference in the rates for cardiovascular abnormalities reached a significant level in Cameron.

The differences in laboratory findings are recognized to be clinically transient and must be evaluated in terms of clinical circumstances. They do not suggest a pattern of association to fluoride effects.

The difference between the age-corrected death rates of the two study areas is not statistically significant.

No clinically significant physiological or functional effects resulted from prolonged ingestion of water containing excessive fluoride, except for dental fluorosis.

SUMMARY

A 10-year study of 116 people in Bartlett and 121 in Cameron, Tex., was conducted to determine if prolonged exposure to fluoride in the water supply of Bartlett had produced detectable physiological effects.

Bartlett's water contained about 8 p.p.m. F until 1952 when an experimental defluoridation unit was installed, reducing the fluoride content to approximately 1.2 p.p.m. F. Cameron was the control area with 0.4 p.p.m. F.

The participants, aged 15 to 68 in 1943, were chosen at random from persons who had resided in the respective communities for at least 15 years. The average length of floride exposure in 1953 was 36.7 years.

In 1943 the investigators took medical histories and gave each participant a medical, X-ray, and dental examination. In 1953 this procedure was repeated for all the original participants (including the removed) except the 18 deceased and 10 of the living.

No significant differences between the two towns were observed, except for a slightly higher rate of cardiovascular abnormalities in Cameron and a marked predominance of dental fluorosis in Bartlett.

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The study was facilitated through the cooperation of the Texas State Health Department; Milam County Health Unit, Texas; Bell and Williamson Coun-

Table 10.—*Prevalence of abnormal laboratory findings, 1953 (removed participants)*

Laboratory Determination	Bartlett		Cameron	
	Number Examined	Number Abnormal	Number Examined	Number Abnormal
Hemoglobin	11	5	20	4
Hematocrit	10	0	20	1
Red blood count	11	0	18	3
White blood count	11	1	20	6
Differential count				
Neutrophiles	11	2	20	3
Lymphocytes	11	6	20	10
Eosinophiles	11	0	20	2
Sedimentation rate	10	5	20	2
Blood calcium	0	0	0	0
Serology (S.T.S.)	5	0	7	0
Urine albumin	13	0	19	0
Urine glucose	13	0	19	0

ties Health Unit, Texas; Scott and White Clinic of Temple, Texas; and the local physicians and dentists of Bartlett and Cameron. Two regional offices of the Public Health Service gave support to the study: Region VIII, Dallas, Texas, by providing transportation and equipment; Region IX, Denver, Colorado, by lending two assistants to the medical-dental team in 1953. The authors are indebted to W. T. Carrigan, Office of the Director, National Institutes of Health, for valuable editorial assistance.

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TABLE 11.—*Prevalence of abnormal laboratory findings, 1943 and 1953 (resident and removed participants)*

Laboratory Determination	Year	Bartlett			Cameron		
		Number Examined	Number Abnormal	Rate, %	Number Examined	Number Abnormal	Rate, %
Hemoglobin	1943	116	34	29.3	121	37	30.6
	1953	90	25	27.8	103	30	29.1
Hematocrit	1943	—	—	—	—	—	—
	1953	89	5	5.6	102	8	7.8
Red blood count	1943	116	25	21.6	121	24	19.8
	1953	91	6	6.6	103	5	4.9
White blood count	1943	116	17	14.7	121	5	4.1
	1953	89	12	13.5	102	13	12.7
Differential count							
Neutrophiles	1943	71	15	28.1	71	6	8.5
	1953	89	25	23.0	102	16	15.7
Lymphocytes	1943	71	2	2.8	71	1	1.4
	1953	89	41	46.1	102	46	45.1
Eosinophiles	1943	71	0	0.0	71	0	0.0
	1953	89	6	6.7	102	16	15.7
Sedimentation rate	1943	—	—	—	—	—	—
	1953	89	36	40.4	103	24	23.3
Blood calcium	1943	—	—	—	—	—	—
	1953	79	9	11.4	66	7	10.6
Serology (S.T.S.)	1943	71	2	2.8	71	3	4.2
	1953	89	2	2.2	102	2	2.0
Urine albumin	1943	115	3	2.6	121	10	8.3
	1953	90	5	5.6	104	12	11.5
Urine glucose	1943	115	2	1.7	121	4	3.3
	1953	90	0	0.0	104	1	0.9

Glossary of Ranges, Standards, and Definitions of Abnormalities

Determination	Defined Range	Reference
	Laboratory	
Hemoglobin		12
Male	14–16 mg %	
Female	12–14 mg %	
Red blood count		12
Male	4.6–6.2 per cu mm	
Female	4.2–5.4 per cu mm	
White blood count	5,000–10,000 per cu mm	12
Differential	%	12
Neutrophiles	50–65	
Lymphocytes	25–35	
Monocytes	4–10	
Basophiles	0–2	
Eosinophiles	0.5–4	
Abnormal cells	Reported as observed	
Hematocrit		12
Male	40–54 vol. %	
Female	37–47 vol. %	
Sedimentation rate (corrected)		12
Male	0–9 mm/hr	
Female	0–20 mm/hr	
Blood calcium	9–11 mg %	13
Urine	Reported as observed (qualitative)	
Albumin		
Glucose		
	Clinical Abnormalities Defined	
Blood pressure		14
Maximum normal for study	150/99	
Systolic hypertension	151 mm Hg & over	
Diastolic hypertension	100 mm Hg & over	
Abnormal pulse pressure in presence or absence of hypertension	75 mm Hg or greater	
Cardiovascular	Presence of arteriosclerosis alone or with decompensation, diastolic murmurs, abnormal pulse pressure, aneurisms, heart block, or other abnormal cardiac findings including hypertension. (For the purpose of the study, hypertension alone is recorded as an abnormality of blood pressure.)	14
Thyroid	When grossly enlarged or palpable. When nodules are present, regardless of size, recorded with tumors and/or cysts.	
Eyes	Cataract and/or lenticular opacity, recorded as observed. Opacity or cataract which developed in second eye since examination in 1943 is designated as one person, but as an increase.	
Ears	Air conduction or bone conduction decreased auditory acuity, but not deafness. Decreased acuity associated with excess cerumen alone not included.	
Gall bladder	Gall stones only when demonstrated by x-ray	
Renal calculi	Renal calculi when demonstrated by x-ray, history of calculi with urine positive for red and white cells, surgical removal, or passage of calculi. (Red blood cells in urine of young females not considered abnormal without supporting CVA tenderness, x-ray or other positive evidence.)	
Prostate	Nodular prostate recorded with tumors and/or cysts.	
Dental fluorosis (mottled enamel)	Presence of very mild through severe fluorosis as determined by Dean's criteria.	15
Other physical examination findings	Recorded as observed.	

Glossary of Ranges, Standards, and Definitions of Abnormalities—Continued

Determination	Defined Range	Reference
	X-Ray	
Bone	For purpose of the study, x-rays of the lumbar spine, sacrum, pelvis, trocanters, and proximal third of the femurs are used for comparisons.	
Hypertrophic bone change	When alone or in combination with bone spurs.	
Increased bone density	When alone or in combination with coarse trabeculation.	
Coarse trabeculation	When alone and not in combination with increased bone density.	
Other bone changes		
Fractures	Recorded as observed	
Osteoporosis	Recorded as observed	
Spurs	When not associated with hypertrophic change	

ORAL ASPECTS OF EXCESSIVE FLUORIDES IN A WATER SUPPLY

By Eugene R. Zimmermann, Nicholas C. Leone, and Francis A. Arnold, Jr.

A 10-year (1943–1953) study of the systemic effects of human ingestion of excessive fluorides in a water supply has been reported recently (*1*). Bartlett, Tex., with a water supply containing approximately 8.0 p.p.m. fluoride (F) was the study area and Cameron, Tex., with about 0.4 p.p.m. fluoride (F) in its water system was the control area. In 1943, residents of Bartlett and Cameron were selected for study if they had lived in their respective areas for a minimum of 15 years and had been relatively continuous consumers of the municipal water supplies. The subjects were not required to have been born in Bartlett or Cameron or to have resided in either town during their tooth formative periods. Each subject received a medical and dental examination.

Table 1.—*Distribution of participants by locality, age, sex, and number of continuous residents, based on 1953 ages*

Age group, years	Mean age, years	No. of persons			Continuous residents*	
		Male	Female	Total	No.	Per cent
			Bartlett			
Under 45	35.7	1	7	8	7	88
45-54	48.7	6	15	21	4	19
55-64	60.6	10	19	29	0	0
65 and over	70.6	6	12	18	0	0
All ages	57.1	23	53	76	11	15
			Cameron			
Under 45	36.7	2	7	9	5	56
45-54	49.9	9	18	27	11	41
55-64	59.5	13	17	30	3	10
65 and over	68.9	4	10	14	1	7
All ages	55.3	28	52	80	20	25

*Participants born and residing in respective localities during period of tooth formation.

In 1953, all of the available original subjects were reexamined. The physical examinations included a routine oral examination by a physician; two-thirds of the subjects were also examined by a dentist. At the end of the study period the subjects, on the average, had been living in Bartlett for 37 years and in Cameron 38 years. Over 75 percent of them began residence in their respective communites after the age of 6 and 60 percent after the age of 14. The majority have continued to live in their respective communities since the initial examination in 1943.

The dental examination was but one phase of the comprehensive medical survey of the subjects, and its purpose was to determine by clinical and roentgenographic methods if excessive fluoride ingestion over a long period of time was associated with changes in the oral tissues. Only the dental observations for those participants who had been examined in 1943 and again in 1953 by a dental officer are presented in this paper.

METHODS

In 1943, 116 persons in Bartlett and 121 in Cameron were examined for dental caries experience, dental fluorosis and periodontal disease. In 1953, 76 of the original Bartlett subjects and 80 of the initial Cameron group were reexamined dentally; these people constituted essentially all of the resident group. The sex ratios of the subjects were approximately the same in the two study groups (table 1). In both examinations mouth mirrors and explorers and artificial illumination were used; dental caries experience was expressed by the DMF (*2*) rate and dental fluorosis by Dean's index (*3*). In 1953, gingivitis was assessed by the PMA index (*4*). In addition, 14 periapical roentgenograms were taken of all examined subjects except two persons in each groups. These films were evaluated for alveolar bone loss according to the method of Stahl, Wisan and Miller (*5*) by four dentists independently (E. R. Zimmermann, E. M. Benjamin and H. R. Stanley, Jr., of Bethesda, Md., and B. A.

Williamowsky of Silver Spring, Md.). The classification of alveolar bone resorption was based on the height of alveolar crests in relation to adjacent roots. Alveolar bone loss was designated as "mild" when the majority of crest-root relationships seen on roentgenograms showed that resorption was limited to the coronal quarter of the root length, "moderate" when resorption was within the second quarter of the root length, and "severe" when resorption was within the apical half of the root length. Resorption was also classified as "horizontal" or "vertical" according to the predominant type.

A method for classifying calculus by degree of severity was devised for the 1953 examination. Visually detected supragingival calculus was considered "slight" if two or more teeth had a surface deposit less than 1 mm. in vertical height, "moderate" if the accumulation was 1 mm. or more in height, but less than "bridging," and "severe" if two or more teeth were bridged by the accretion. If only infragingival calculus was observed, the deposit was designated as "slight."

OBSERVATIONS

Fluorosis.—All persons with fluorosis in Bartlett or Cameron had been residents in a fluoride area during the period of calcification of their permanent teeth. As can be seen in table 1, however, comparatively few of the Bartlett subjects in this study were born in Bartlett or reared in that community while the permanent teeth were forming. This accounts for the low prevalence of fluorosis in this group. Ten of 45 nonedentulous individuals in Bartlett (22.2 percent) and 1 of 64 in Cameron (1.6 percent) had positive signs of fluorosis in 1953. Although different dentists examined the subjects in 1943 and 1953, there was agreement in 91 percent of the "positive" cases. As would be expected, fluorosis was neither more prevalent nor more severe among the individuals after this 10-year period of exposure. Since the fluorosis pattern of these persons had been established during the first 8 years of life, they were not "at risk" for fluorosis during the study period.

Decayed, Missing, or Filled Permanent Teeth.—Although this investigation was not designed primarily to study the fluoride-dental caries relationship, it did offer useful information on the 10 year incidence of caries experience in these two groups of people. The DMF rates of those examined in 1943 and 1953 are presented in table 2. Bartlett's DMF rate was lower than Cameron's in 1943 but higher in 1953. The latter was due principally to an increased number of missing teeth in Bartlett. At the end of the study period, 47 percent of the tooth loss in Bartlett and 25 percent in Cameron was attributed by the individuals themselves to periodontal disease; 5 percent of the tooth mortality in Bartlett and 1 percent in Cameron was ascribed to general health considerations, trauma and appearance. If the teeth lost through periodontal disease and causes other than caries were excluded from the 1953 DMF rate, the caries experience of the subjects in Bartlett would be 33 percent lower than was that of the subjects in Cameron.

Table 3.—*Amount of gingivitis per person (PMA units) in the nonedentulous Bartlett and Cameron participants*

Age group, years	Bartlett: No. of persons	Bartlett: No. of gingival units affected: P	M	A	total	Bartlett: Mean units per person	Bartlett: S.E. of the mean	Cameron: No. of persons	Cameron: No. of gingival units affected: P	M	A	total	Cameron: Mean units per person	Cameron: S.E. of the mean	Significant difference P=.05
Under 45	7	65	62	23	150	21.43	10.52	9	70	57	21	148	16.44	4.86	no
45-54	15	175	159	35	369	24.60	4.86	26	204	173	50	427	16.42	2.32	no
55-64	15	116	110	31	257	17.13	4.38	19	221	187	90	498	26.21	4.44	no
65 and over	8	87	83	20	190	23.75	5.95	10	127	106	27	260	26.00	4.50	no
All ages	45	443	414	109	966	21.47	2.75	64	622	523	188	1333	20.83	1.92	no

Table 2.—*Dental caries experience, excluding third molars, of the same Bartlett and Cameron participants examined in 1943 and 1953*

Age group, years (by 1953 ages)	No. of persons	DMF rate*: 1943	DMF rate*: 1953	No. of missing teeth attributed to dental disease†: 1943	1953	diff.	increment/person
				Bartlett			
Under 45	8	5.6	10.5	10	11	1	0.1
45-54	21	8.1	13.9	107	214	107	5.1
55-64	29	16.4	19.3	300	430	130	4.5
65 and over	18	19.6	20.6	315	354	39	2.2
All ages	76	13.7	17.2	732	1009	277	3.6
				Cameron			
Under 45	9	6.2	10.4	16	25	9	1.0
45-54	27	12.4	14.7	80	124	44	1.6
55-64	30	17.8	19.6	348	399	51	1.7
65 and over	14	14.9	19.5	134	211	77	5.5
All ages	80	14.2	16.9	578	759	181	2.3

*Number of decayed missing and filled permanent teeth per person.

†Periodontal disease or dental caries.

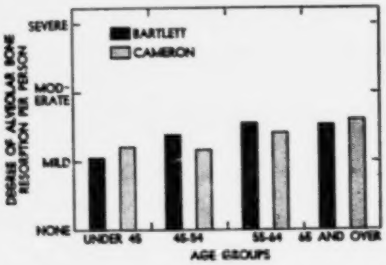
Periodontal Disease.—In 1943, no important differences in the health of the periodontal tissues were observed between the Bartlett and Cameron residents. In 1953, the clinical (table 3) and roentgenographic (illustration) evaluations of the subjects' periodontal condition show that the amount of gingivitis and alveolar bone resorption did not differ significantly between the two groups. When measured clinically by another quantitative method (*6*), the results again were similar.

Other Oral Conditions.—No important differences in the prevalence of oral tori, leukoplakia, lichen planus, soft tissue color abnormality, calculus, pulp stones, horizontal or vertical alveolar bone resorption, periapical rarefaction, condensing osteitis and dentigerous cysts were found between the Bartlett and Cameron subjects (table 4). The difference in number of examined persons in this table is due to the exclusion of edentulous individuals or those for whom roentgenograms were not made from the calculations of certain prevalence rates.

DISCUSSION

When the composition of the sample is considered, it is not surprising that Bartlett's DMF rate is similar to that in Cameron. There were comparatively few continuous residents in the Bartlett study group; only 11 of 76 individuals were born in Bartlett or were reared there during the period of tooth formation. Consequently, the relatively low DMF rate of the continuous residents forms only a small part of the total DMF rate in Bartlett.

At the end of the 10-year study period, more teeth per person were missing in Bartlett than in Cameron (table 2), but this difference was not statistically significant. There were more full-mouth extractions in Bartlett than in Cameron. A larger segment of the tooth mortality rate was attributed to periodontal disease



Degree of alveolar bone resorption per person, by age groups, in the 45 Bartlett and 64 Cameron participants with natural dentitions.

and causes other than caries by the Bartlett subjects than by the Cameron residents. In contrast, however, the clinical examinations in 1943 and in 1953 and the roentgenographic evaluations in 1953 showed similar periodontal conditions in both groups; these observations do not suggest that more teeth were lost in Bartlett because of periodontal disease. A possible explanation of the greater tooth loss in Bartlett is a difference in the availability of dental care and in dental health education. In Cameron, there were six dentists practicing in 1943 and four in 1953. In Bartlett, there was only one dentist practicing in 1943, and he was practicing only on a part time basis in 1953. On a per capita basis, almost twice as much dental care was available in Cameron as in Bartlett.

The data in this study do not support the statements that residents of fluoride areas have a greater prevalence and severity of periodontal disease (*7*, *8*) and pulp stones (*8*) than those of nonfluoride areas. Both clinical and roentgenographic evaluations, performed without reference to each other, indicated that the periodontal health of the Bartlett and Cameron subjects was approximately the same. A statistical comparison of these observations was made, and a positive and linear relationship between them resulted.

Approximately 78 percent of the Bartlett residents and 75 percent of the Cameron subjects were found to have calculus. A larger percent of the Bartlett subjects had an amount classified as "slight" and "severe"; more Cameron residents were found with "moderate" calculus. In general, age and amount of calculus were directly proportional; a greater amount of calculus was observed on the teeth of older subjects.

SUMMARY

1. A 10-year (1943–1953) study of two groups of people residing in Bartlett (8.0 p.p.m. fluoride (F)) and Cameron (0.4 p.p.m. fluoride (F)), Tex., was conducted to determine if the prolonged use of excessive fluorides in a water supply was associated with physiological changes in body tissues. The oral aspects of this study have been presented in this report.

2. One hundred and fifty-six participants, 76 in Bartlett and 80 in Cameron, were examined in 1943 and in 1953 for dental caries experience, gingivitis, dental fluorosis, and other types of oral pathology.

3. As was expected, dental fluorosis was significantly higher in Bartlett than in Cameron. The amount of gingivitis, alveolar bone resorption, dental caries experience, calculus and the prevalence of leukoplakia, lichen planus, soft tissue color abnormality, pulp stones, horizontal and vertical alveolar bone resorption, periapical rarefaction, condensing osteitis, and dentigerous cysts were examined statistically and no significant difference was found between the Bartlett and Cameron residents.

TABLE 4.—*Prevalence of certain dental conditions among adults of Bartlett and Cameron, Tex., 1953*

	Bartlett			Cameron			
	Number examined	No. with condition	% (rate)	Number examined	No. with condition	% (rate)	Significant difference P=.05
Clinical examination							
1. Calculus	45			64			
a. slight		17	37.8		18	28.1	no
b. moderate		9	20.0		23	35.9	no
c. severe		9	20.0		7	10.9	no
2. Tori palatinus and mandibularis	76	12	15.8	80	20	25.0	no
3. Soft tissue pathology							
a. Leukoplakia	76	1	1.3	80	4	5.0	no
b. Lichen Planus	76	0	0	80	2	2.5	no
c. Color abnormality	76	4	5.3	80	8	10.0	no
Roentgenographic examination							
1. Pulp stones	43	8	18.6	63	15	23.8	no
2. Alveolar bone resorption							
a. Horizontal type	43	41	95.4	63	57	90.5	no
b. Vertical type	43	0	0	63	2	3.2	no
3. Periapical rarefaction	43	4	9.3	63	6	9.5	no
4. Condensing osteitis	74	5	6.8	78	6	7.7	no
5. Dentigerous cyst	74	0	0	78	1	1.3	no

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A ROENTGENOLOGIC STUDY OF A HUMAN POPULATION EXPOSED TO HIGH-FLUORIDE DOMESTIC WATER (A 10-Year Study)

By Nicholas C. Leone, Clyde A. Stevenson, Theodore F. Hilbish, and Merrill C. Sosman

INTRODUCTION

Roentgenological manifestations of fluoride effects in human bones occur rarely and are less often recognized. When bone changes due to fluorides were first described by Roholm (*13*) in 1937, they were gross changes and, without question, most unusual. Similar bone changes ascribed to fluoride effects have been reported by a number of investigators under various circumstances. In most instances the number of cases was limited; there has been a tendency to stress the bizarre, attention has been directed to individuals with gross physical change or roentgeneographic evidence of marked change; and the lack of bone change in other members of the same population group with comparable risk of exposure has not been emphasized or reported (*2, 3, 6, 8, 9, 10, 12, 13, 15*).

Many American communities have long used domestic water supplies containing natural fluorides ranging from 1 to 6 p.p.m. (parts per million) F, and in a few instances, higher concentrations, but rarely have roentgenologists observed or reported bone changes known to be associated with fluorides from this source (*13*).

More recently, the introduction of fluorides into water supplies as a public health measure has created new interest in fluoride effects. Unusual changes, described earlier in cryolite and rock phosphate workers have, by implication, typified the "effects of fluoride exposure." These findings have created a bias in the interpretation of the physiologic fluoride effects—a bias that has pervaded the evaluation of data concerning domestic water supplies containing but trace amounts of natural or mechanically added fluoride.

The primary object of this report is to present the roentgenographic findings of a ten-year study of 237 persons, approximately one-half residing in a high-fluoride area (Bartlett, Tex.), 8 p.p.m. F in drinking water, and the other half residing in a control area (Cameron, Tex.), where the water supply contained 0.4 p.p.m. F, and to describe the findings that might be ascribed to prolonged fluoride ingestion. Comparative evidence of bone change from a long term large animal study is also presented.

PARTICIPANTS, METHODS AND PROCEDURES

Roentgenograms made in 1943, and repeated on the same individuals with matching views 10 years later, enabled comparative study of the individuals in each group with themselves and of those in a high-fluoride group with those in a low-fluoride group. The roentgenographic findings were also correlated with the individual medical histories, physical examinations, and laboratory data to evaluate the presence or absence of any detectable effects in the individual in the groups, or in the various age categories.

Participants.—In the groups studied there were 116 participants from a high-fluoride area and 121 from the control area, a total of 237 persons. The period of study was 10 years. The average (natural) fluoride content of the water was 8 p.p.m. F in the high-fluoride area and 0.4 p.p.m. F in the control area. The average length of exposure was 37 years in the high-fluoride area and 38 years in the control area. The participants were all white, and their ages in 1943 ranged from 15 to 68 years. They were originally selected on the basis of minimum residence of 15 years in the respective towns.

The procedure, method of handling the data, and the details of the study have been reported in an earlier paper (7).

In the 10-year interval between 1943 and the 1953 followup studies, 47 original participants moved from the immediate study areas. The number that left each town was almost equal—22 from Bartlett and 25 from Cameron, predominantly of the younger age groups. These "removed" participants were located, and similar roentgenographic studies were made in all but 10 cases.

Methods and Procedures.—The roentgenograms were made at the Scott and White Clinic, Temple, Tex., under the immediate supervision of the same Chief of Service, in 1943 and again in 1953. A 500 ma. roentgenographic unit with rotating anode tube was used on both occasions. Emphasis was placed on bone detail. Routinely, anteroposterior roentgenograms of the dorsal and lumbar spine and the pelvis, showing the proximal third of each femur, were made for each patient in 1943 and repeated in 1953. When a question of unusual findings arose, or when a participant was considered to have increased bone density, coarsened trabeculation or other important bone change, a roentgenographic bone survey was made, consisting of the following views: a lateral skull (stereo), cervical spine; left upper arm, forearm, and hand; and right femur, lower leg, and foot. Additional views were taken when indicated.

Of the 47 "removed" participants, only 4 had left the State of Texas, and it was therefore possible to transport the greater number of these participants to the Scott and White Clinic, where they were examined in the same manner, and with the same equipment, as the other participants. No attempt is made to evaluate the "removed" participants separately, because no comparable roentgenographic differences were noted in these persons from either area.

Roentgenographic Evaluation.—The findings reported in each case have been limited to an evaluation of anteroposterior views of the lumbar spine, sacrum, pelvis, trochanters and proximal one-third of the femurs. Films made in 1943 were compared with similar views of the same region, of the same individuals, repeated in 1953. The regions selected for roentgenograms represent those in which the earliest or most definite manifestations of fluoride effects might be seen if present (*2, 4, 13*). Moreover they best show the roentgenographic characteristics under discussion and permit more uniform evaluation.

Interpretation of the findings was based on those bone changes which have been generally designated to be found in association with exposure and ingestion of excessive amounts of fluorides—i.e., increased bone density, coarsened trabeculation, hypertrophic change, and ligamentous calcification (*9, 10, 12, 13*). A 1 mm. metallic "step wedge" was employed as a guide to the interpretation of bone densities, but this increased the difficulty of unbiased evaluation and was therefore disregarded as an instrument of measure. Differences attributable to technique or film storage were taken into account, but little storage change occurred, and no difficulties of interpretation were encountered from this source. Bone spurs unassociated with hypertrophic change and osteoporosis associated with aging and postmenopausal syndrome were also considered. As used in this paper, "osteoporosis" is defined as a deficiency in organic protein matrix.

while "osteomalacia" is a calcium deficiency or inability to deposit calcium salts in the bone matrix. Classical examples of osteoporosis are Cushing's disease or postmenopausal changes in the spine and pelvis. "Osteomalacia" is exemplified by the osseous changes which follow severe renal disease with acidosis, steatorrhea or vitamin D intoxication. Evaluations were made on the basis of age, sex, activity, study area, and elapsed time.

Bone density changes, as described here, refer to increased or decreased density in the presence or absence of coarsened trabeculation. In reference to hypertrophic change, only those cases recorded as modarte (2+) severe-limited (3+), or severe-generalized (4+) have been considered. Some hypertrophic changes, particularly in aged persons, is a normal finding and, when moderate, was not considered as a possible fluoride effect. Roentgenographic evaluation included a correlation with individual histories and physical examinations. Bone fracture histories, arthritis and systemic conditions which might be associated with roentgenographic findings were also tabulated. There were no significant differences in these conditions within the study groups (7).

RESULTS

As shown in table 1, a limited number of participants from both Bartlett and Cameron showed some degree of roentgenographic bone change, but, in general, these changes were minimal. There was a larger number with no observable change in the 10-year interval, a fact disregarded or unreported in most fluoride studies (*1, 2, 8, 10, 12, 13*). It was therefore evident that high concentrations of fluoride in drinking water did not uniformly produce bone change of a type detectable in even an appreciable portion of the participants living in the high-fluoride area. Only one new case of increased bone density was found in the latter group at the end of the 10-year period.

TABLE 1.—*Bartlett-Cameron fluoride study. Roentgenographic bone changes in participants studied in both 1943 and 1953*

Abnormality	Bartlett Number Studied: 89		Cameron Number Studied: 101	
	1943	1953	1943	1953
Bone density changes*	16	17	4	4
Increased		14†		3
Decreased‡		3		1
Osteoporosis	4	5	2	10
Coarsened trabeculation (without increased bone density)	12	14	2	3
Hypertrophic change§	3	3	1	1

* Increased or decreased bone density with or without coarsened trabeculation, excluding osteoporotic change.

† Includes one new case of increased bone density in 1953.

‡ Decreased bone density, but not osteoporosis.

§ Only cases with "severe-limited" (3+) and "severe-generalized" (4+) are included.

The absence of unusual roentgenographic evidence of bone or other physiologic change on long exposure in a high-fluoride area is best substantiated through the histological and chemical examination of tissues from one of the study group participants, from Bartlett, who died shortly after the 1953 examinations. The patient, a female, aged 79, with a history of 52 years' residence in the high-fluoride area, died from a massive cerebral vascular accident. A complete autopsy was performed and bone was obtained for histologic examination and chemical analyses. The roentgenographic examinations in 1943 and 1953 showed a moderate (average) degree of increased bone density with some coarsened trabeculation. Chemical analysis of the bones showed approximately six times the fluoride (F) content (0.6 mg. percent) of the same bone from individuals from nonfluoride regions. There were no significant histologic changes that could be linked to fluoride exposure.

Bone Density, Coarsened Trabeculation and Osteoporosis.—Of the original Bartlett participants, 16 exhibited roentgenographic bone changes in varying degrees in 1943 that were considered of interest to this study. At the end of the 10-year period (1953) 9 of the 16 showed no further bone change, 4 showed an increase in bone density, and 3 a decrease in density toward a "normal" appearance. Only one new case of increased bone density was recorded. The latter is a finding of medical interest, because if prolonged fluoride exposure induces specific or generalized bone changes, they would be expected to appear in various degrees in the majority of persons exposed. Of the cases designated as "increased density," only two could be considered frank abnormalities. None approached the extent and degree of change described by Roholm and others (*2, 9, 10, 12, 13*). Roholm (*13*) differentiates his fluoride bone changes into "3 phases of the same osteosclerosis process, each over-lapping the next without sharp boundary, e.g. First phase—the changes are observed in pelvis and columna, but are doubtful or absent elsewhere."

In Cameron, the control area, there were four cases of increased density, two cases of increased coarsened trabeculation, and eight new cases of osteoporosis during the 10-year period, as compared with one new case of osteoporosis in Bartlett. During the same period four Bartlett participants showed increased coarsened trabeculation without increased bone density.

Increased bone density occurred predominantly in persons over the age of 45. It ranged from a slight "fuzziness" or "ground-glass" appearance, without cortical bone thickening or change in the bone marrow space, to a definite "frosted glass" appearance with clearly visible coarsened trabecular markings, an equivocal increase in cortical thickness, and a slight relative decrease in the bone marrow space. There was minimal periosteal thickening in a few instances, and an occasional case showed osteophytes or localized calcification of ligamentous attachments—a common finding in any older age group.

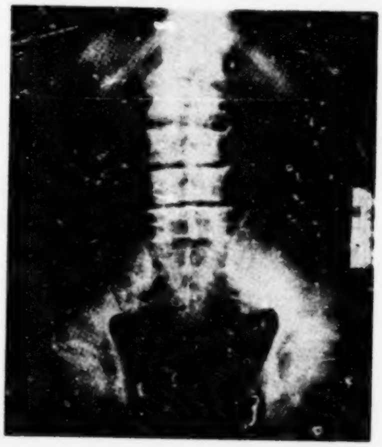

FIGURE 1.—*Case I. Roentgenogram shows the most pronounced increased bone density and coarsened trabeculation in the series, 1943.*

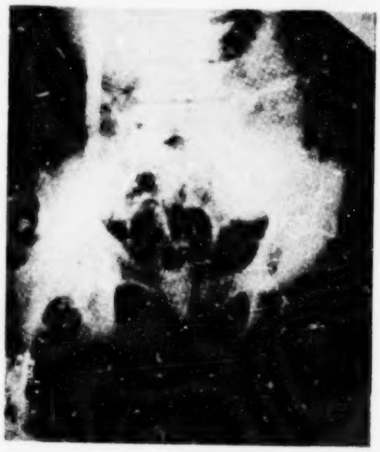

FIGURE 2.—*Case I. The same participant 10 years later, 1953.*

Decreased bone density in those who earlier showed an increased density in 1943 is of great interest, as seen through a transition from a dense bone structure to less dense appearing bone 10 years later.

REPORT OF CASES

The character and degree of roentgenologic change observed is best illustrated by the presentation of representative cases.

Increased Density and Coarsened Trabeculation.—The most pronounced change in terms of increased bone density and coarsened trabeculation in the study series is illustrated in figures 1 and 2.

Case 1.—The patient is a 72-year-old white male, resident of Bartlett for 33 years. On physical examination he was found to have hypertension (blood pressure 190/100), generalized arteriosclerosis, hypertrophic change of the hands (patient has been a rope handler all his life), indurated prostate, right inguinal hernia with a large segment of bowel incarcerated in the scrotum, varicose veins and bilateral increased lenticular opacities. Blood and urine studies, including serum calcium, acid and alkaline phosphatase. were not remarkable.

Roentgenographic Findings.—The roentgenographic change in this particular patient seems to be that of an increase in the total density of the bone throughout the entire lumbar spine and pelvis. The change also involves a coarsening of the trabeculae, such as we see in Paget's disease. The process seems to lie halfway between the normal trabecular pattern in density and the thickened denser trabeculae in classic Paget's disease. The process is uniform through all bone and not localized or more exaggerated in certain areas, such as we ordinarily see with Paget's disease. A similar film of the lumbar spine, pelvis, and hips of this same patient, taken 10 years later (1953), shows no apparent change in the amount or the character of this abnormality.

Normal Male Pelvis.—To permit better comparison of the cases under discussion, a half pelvis view of a 45-year-old "normal male" as compared with a half pelvis view of the patient discussed in case 1 is shown in figure 3.

Average Roentgenographic Bone Change (Minimal Increased Density with Coarsened Trabeculation).—The roentgenographic bone change seen in the

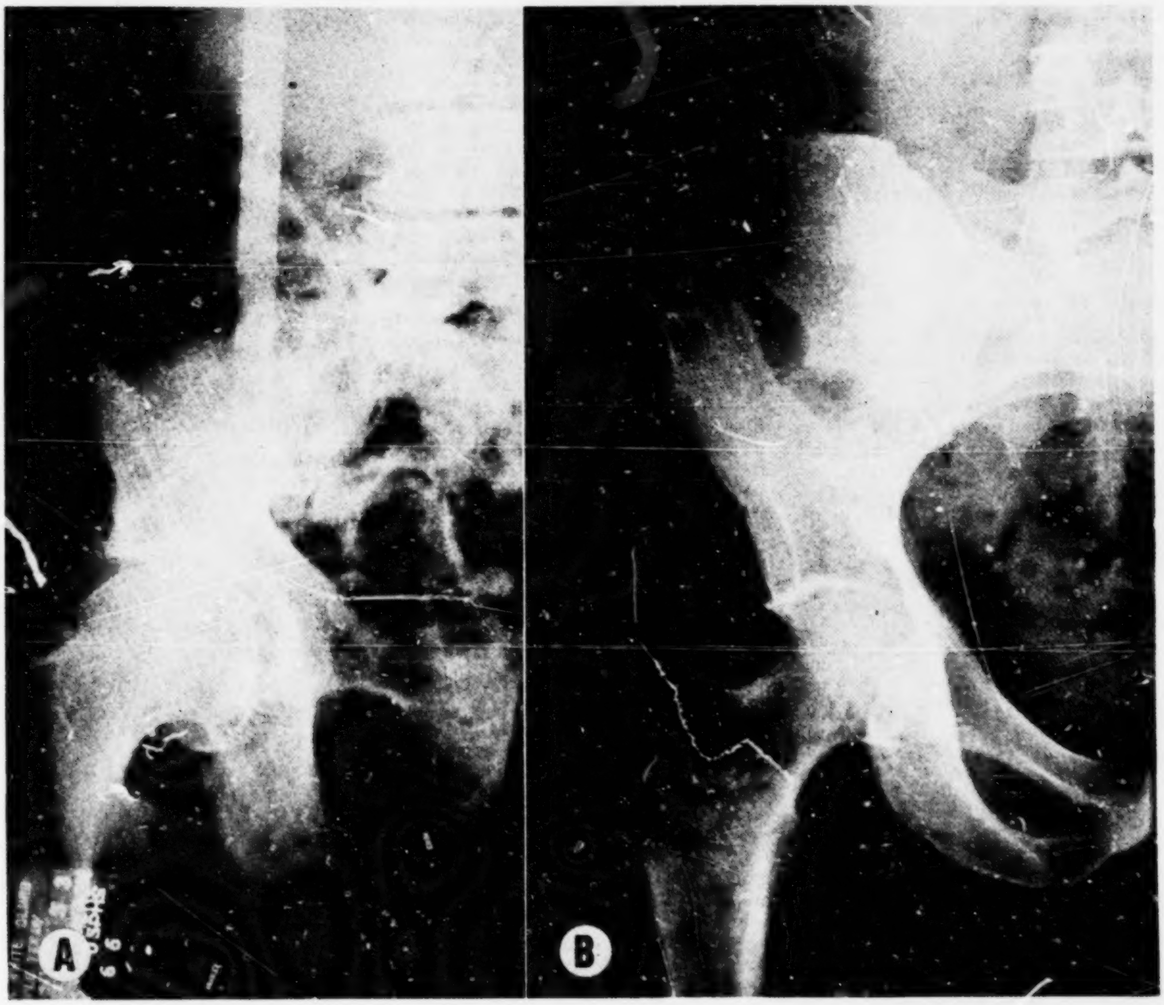

Figure 3.—*Case I.* (A)*Roentgenogram showing the most pronounced changes in the series, compared with* (B) *half-pelvis view of "normal" male pelvis.*

majority of positive cases can be designated as typical of the series (fig. 4 and and 5). These changes, often equivocal, might easily be overlooked.

Case II.—The patient is a 71-year-old white male, resident of Bartlett for 59 years. The medical history is not remarkable except for a serious automobile accident in 1949, resulting in multiple fractures, with subsequent good union, no complications or other residual effects. The patient was mentally alert and physically active and claimed excellent health. On physical examination the patient was found to be in unusually good condition for his age except for 1° induration of the prostate, poor dentition and senile hyperkeratosis. The laboratory findings were not remarkable.

Roentgenographic Findings.—The lumbar spine and pelvis show a slight, but distinct increase in the total density of the bones with coarsening of the trabeculae, most evident in the sacrum and not distinct in any other portion of the pelvic bones. In fact, the trabeculae in the descending rami of the ischia appear to be quite fine and delicate but still with increased density, particularly just below the acetabula. There is not the individual smudging of detail or amorphous appearance such as is seen in cases of metastatic carcinoma of the prostate, but it approaches this type of appearance rather than the thick, dense trabeculae of Paget's disease. There is, in addition, definite ossification of the right sacrotuberous ligament, somewhat more extensive in 1953 than in 1943. There is no change in the degree of density when the 1943 and 1953 films are compared.

Increased Bone Density and Coarsened Trabeculation with a Decrease in Bone Density 10 Years Later.—A case of unusual medical interest is that of a woman (case III) who showed increased bone density and coarsened trabeculation in 1943 with a decrease in bone density to a point more closely resembling "normal" bone 10 years later (fig. 6 and 7). Several such cases were noted in the series.

Case III.—The patient is a 59-year-old white female, resident of Bartlett for 39 years. On physical examination, there was systolic hypertension (200/78) with aortic insufficiency (early diastolic decrescendo murmur), slight, bilateral, air-conduction hearing defect and complete edentia, compensated. Physical examination and laboratory findings were otherwise not remarkable.

Roentgenographic Findings.—Roentgenograms of the lumbar spine, pelvis and femurs taken in 1943 show a rather marked increase in the total density of the bones, particularly evident in the vertebrae, sacrum, and around the sacroiliac joints. There is also a definite coarsening of the trabeculae in that portion of the skeleton visualized.

A remarkable change is apparent in the second series of films, taken in November 1953 (10 years later). The increased density previously noted has decreased appreciably and the trabecular pattern has also lost much of its coarsening. There are small bony spurs or ossifications in the region of the sacrotuberous ligaments, and one sacrospinous ligament, slightly more pronounced at the time of the second examination. It is tempting to conjecture that this patient was still having normal menstrual periods at the time of the first examination (aged 49), and that the change seen is due to postmenopausal osteoporosis, a well known endocrine deficiency pattern. Review of the patient's medical history disclosed menopause at about age 54, with normal menstrual periods at the time of the first examination in 1943.

Fifty-Year Fluoride Exposure without Bone Effects.—An 84-year-old female resident of Bartlett with known exposure to a high level of naturally occurring fluorides for 50 years, and a resident of the high-fluoride area immediately adjacent to Bartlett for the rest of her life (total exposure of 84 years to 4–8 p.p.m.

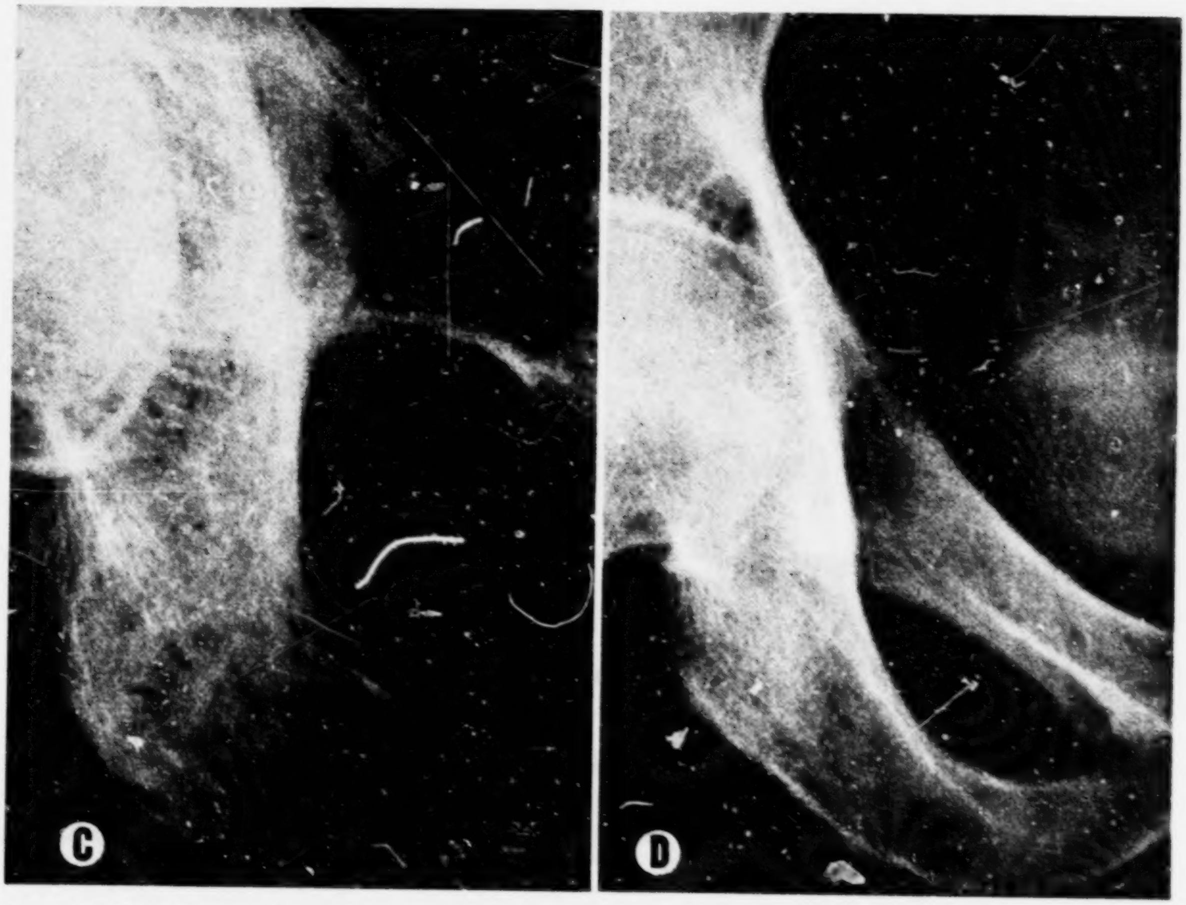

Figure 3.—(C) *Enlarged section of pubic rami from Case I (1953) seen in Figure 3A.* (D) *Enlarged section of pubic rami from "normal" pelvis seen in Figure 3B.*

F) provides an interesting aspect to the claims of gross bone change on long exposure to fluorides. In this case there is no evidence of increased bone density, coarsened trabeculation, or other significant roentgenographic bone changes (fig. 8). This patient was not a participant of the original 1943 study group, but is the mother of one of the participants, and a member of the same household. The case is included to illustrate that a lifetime of exposure to high-fluoride levels does not necessarily produce changes often described as "fluoride bone effects" or produce recognizable bone changes in all of those who are exposed. The patient has enjoyed excellent health all her life, and at the age of 84 remains mentally alert, physically active, and without need of assistance in her daily activities.

ROENTGENOGRAPHIC BONE CHANGE IN CATTLE

Bone changes in cattle, resulting from prolonged ingestion of toxic levels of fluoride (2.2–2.5 mg./kg. body weight) provide incontrovertible roentgenographic proof of the ability of unusually high fluoride levels to produce certain types of change and to stimulate new bone formation. They show, too, the nature and degree of the change. No similar or comparable change was found in any of the human cases in the Bartlett-Cameron series. The Utah State Agricultural College research group has co-ordinated certain aspects of its broad, long-range, large-animal fluoride studies with a part of the Public Health Service fluoride studies in humans to permit parallel comparisons. The following cases are from a large series under study.

Cattle Bones.—The metacarpal bone of a 10-year-old normal control animal (fig. 9, left) is roentgenographically compared with the metacarpal bone of a 10-year-old animal which ingested fluoride at a toxic level for approximately seven years (fig. 9, right).

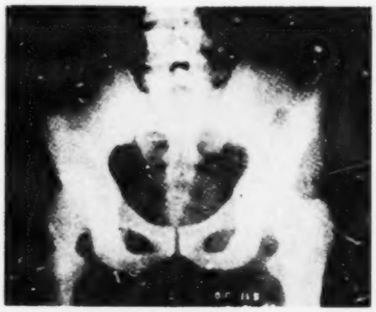

Figure 4A.—*Case II. Roentgenogram shows average degree of increased bone density found in 14 participants in the study series, 1943.*

Roentgenographic Findings.—The bone on the left appears to be of normal density, texture, and shape while that on the right shows a definite increased density, with a ground glass appearance, moderate coarsening of the trabecular pattern, and increased cortical thickening with narrowing of the bone marrow space. There is definite irregular periosteal thickening in the form of new bone, laid down along the shaft. There is no evidence of any disease or condition which could produce the striking abnormality in this bone which simulates the hypertrophic pulmonary osteoarthropathy seen in humans.

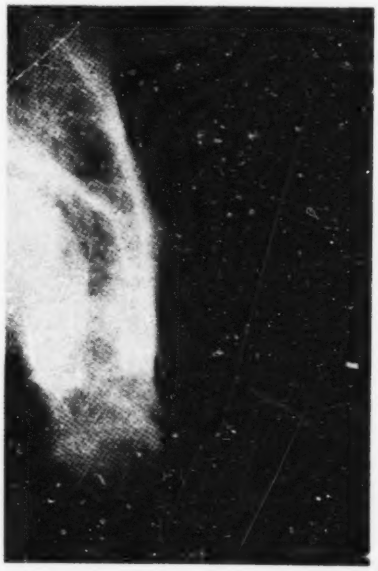

Figure 4B.—*Case II. Enlarged section of pubic rami from figure 4A, 1943.*

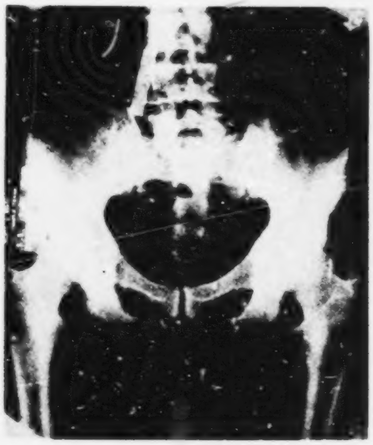

Figure 5A.—*Case II. The same participant 10 years later, 1953, without evidence of further significant change.*

The cattle bone roentgenograms were made at the Scott and White Clinic, Temple, Tex., on the same roentgenographic unit as was used in the Bartlett-Cameron human series. One of the authors carried the refrigerated bones to Temple, Tex., where the roentgenograms were made 2 days after autopsy. Both animals were sacrificed on the same day.

CONCLUSIONS

1. The following types of roentgenographic bone conditions were seen in humans who used a water supply containing excesive fluoride (8 p.p.m. F) for long periods.

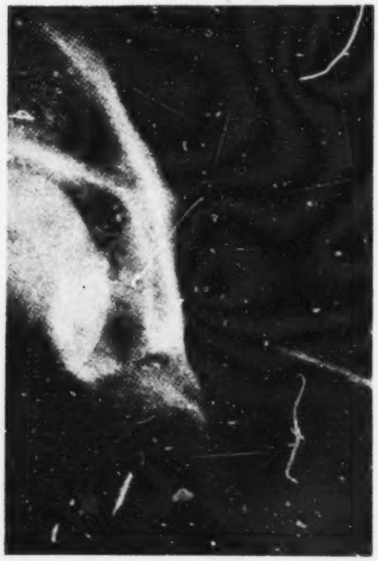

Figure 5B.—*Case II. Enlarged section of pubic rami from figure 5A, 1953.*

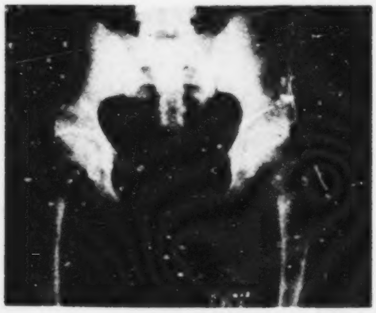

Figure 6A.—*Case III. Roentgenogram shows increased bone density and coarsened trabeculation in a female, 1943.*

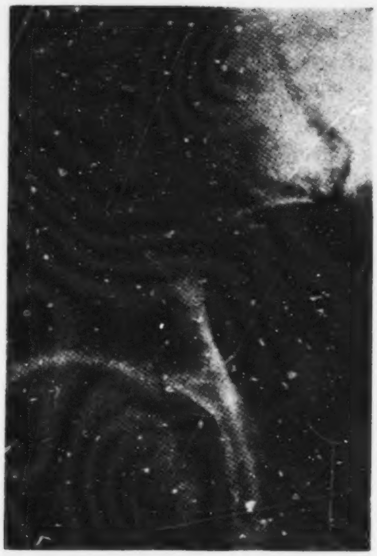

Figure 6B.—*Case III. Enlarged section of pubic rami from figure 6A, 1943.*

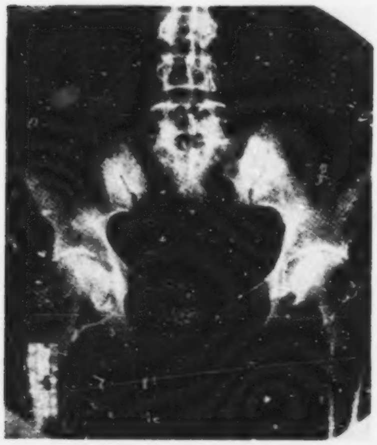

Figure 7A.—*Case III. The same participant 10 years later, 1953, showing a decrease in bone density.*

(a) Increased bone density with or without coarsened trabeculation, with a "ground-glass" appearance.

(b) Coarsened trabeculation, showing lines of stress, without increased bone density.

(c) Increased thickening of cortical bone and periosteum with equivocal narrowing of bone marrow spaces. (This is positively demonstrated in cattle at toxic levels.)

2. The data, based on a study of 237 cases presented on a statistical basis in an earlier paper clearly demonstrate that except for dental mottling, ingestion of water containing fluorides up to 8 p.p.m. produces no deleterious bone changes: no unusual incidence of bone fractures, arthritis, hypertrophic bone changes or exostoses, or interference with fracture healing; no cases of "poker spine" and no evidence of associated functional or systemic effects (7).

3. Excessive fluorides in a water supply may produce roentgenographic evidence of bone changes, but the observable changes—

(a) Occur in only a select few (approximately 10 to 15 percent of those exposed).

(b) Are slight, often difficult to recognize, and in most instances equivocal in degree.

(c) Bear no resemblance to the bizarre findings described in some cases of long exposure to cryolite or rock phosphate dust, nor to those attributed by some investigators to excessive fluorides in domestic water supplies.

(d) Are not associated with other physical findings, except for dental mottling in persons who resided in the high-fluoride area during the tooth formative period (up to 8 years of age).

(e) Cannot be definitely ascribed to excessive fluorides alone.

(f) Do not necessarily occur even though the fluoride content of the bone may be six times that of "normal" bone.

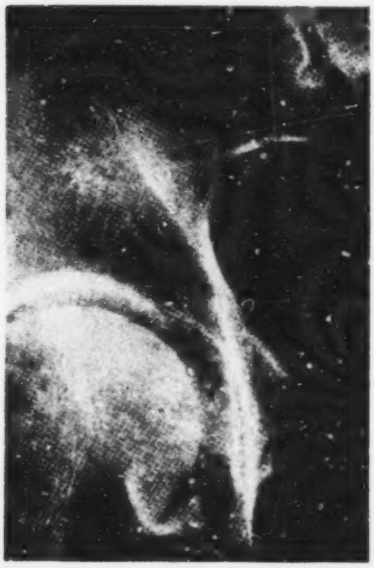

Figure 7B.—*Case III. Enlarged section of pubic rami from figure 7A, 1953.*

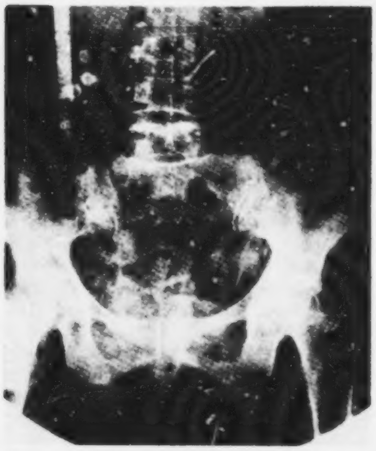

Figure 8.—*Case IV. The pelvis of an 84-year-old female, a life-time resident in a high fluoride area, showing no evidence of increased bone density or coarsened trabeculation.*

4. There is some indication that the ingestion of excessive fluoride in water and the "fluoride effect" of the degree encountered in this study may; on occasion, have a beneficial effect in adult bone, as in counteracting the osteoporotic changes of the aged.

ACKNOWLEDGMENTS

The services of Miss Patricia Geiser, public health nurse officer and member of the survey team, Jacob Lieberman, statistician, Office of Biometry, National Institutes of Health, who aided in the design of the study and evaluation of the data, and E. F. Foegelle, Scott and White Clinic, Chief X-ray technician, were important to the success of this study. We also wish to express our appreciation to the Scott and White Clinic for their participation in the Bartlett-Cameron Study, and for their facilities, made available to the survey group in 1943 and again in 1953.

The interest and cooperation of Drs. Greenwood and Shupe of the Utah State Agricultural College in granting the authors permission to use the roentgenograms of mature cattle bones from one of their study series, for comparison with the roentgenologic human findings, was an important contribution to the success of the study.

A special acknowledgment is made to Dr. H. Trendley Dean, former Director, National Institute of Dental Research, who conceived and initiated the study in its broad aspects.

Public Health Service Examining Teams

1943—Physicians: J. W. Hawkins, M. B. Shimkin, Chester Brown; Dentist: F. A. Arnold, Jr.; Nurses: Christine Mason, Mary Harte; Technician: Stoel Davis.

1953—Physician: N. C. Leone; Den-

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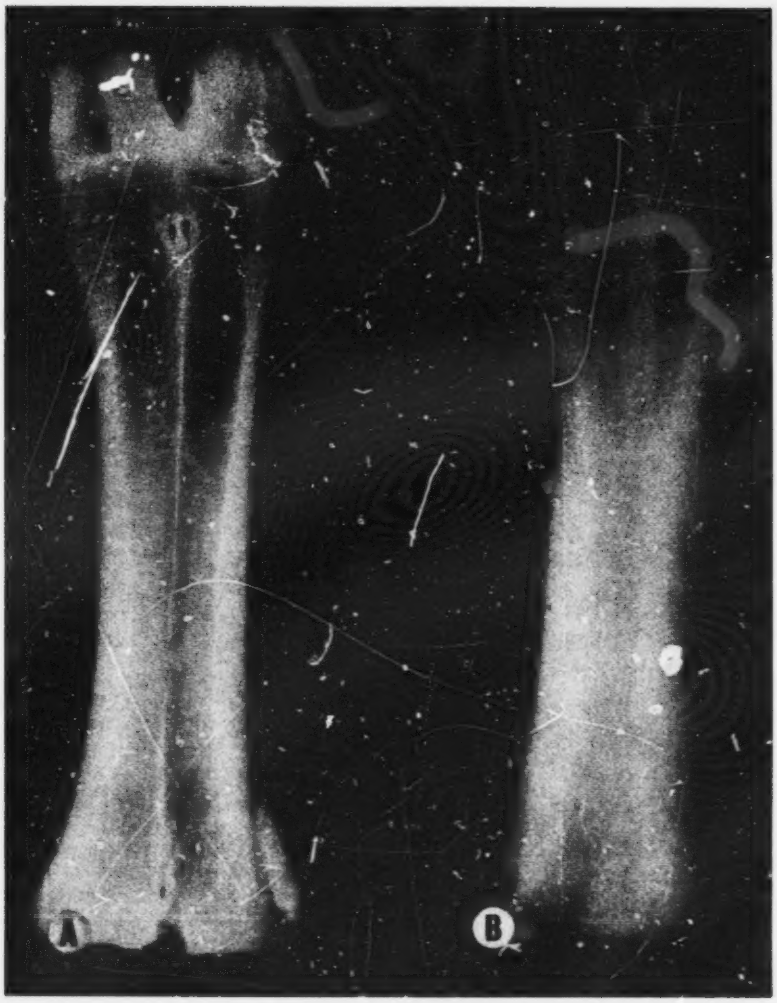

FIGURE 9.—*Bovine metatarsal bones.* (A) *Control animal bone.* (B) *Bone from animal on prolonged toxic fluoride level.*

tist: E. R. Zimmermann; Nurse: Patricia Geiser; Assistants: Vona Harrell, F. D. Baty, Elaine Pickett.

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URINARY EXCRETION OF FLUORIDE FOLLOWING DEFLUORIDATION OF A WATER SUPPLY

By R. C. Likins, F. J. McClure, and A. C. Steere

Previous studies have shown that essentially all absorbed fluoride is eliminated in the urine or deposited in skeletal and dental tissues (*1–3*). That all fluoride deposited in the skeleton is not fixed irreversibly is shown by its mobilization following a reduction in fluoride intake (*1, 3–9*). Brun and his associates (*4*) reported that men who had absorbed fluoride from cryolite dust maintained a high level of fluoride in the urine for as long as 7 years following the period of exposure. Similarly, Largent and Heyroth (*3*) and Largent (*5*) found that urinary excretion of fluoride in excess of the intake continued at a progressively decreasing rate for as long as 2 years after the ingestion of large amounts of fluoride. Blakemore and his coworkers (*6*) observed that in cattle the fluoride content of the urine remained high for some time after they had foraged on pastures contaminated with industrial fluoride dust. Direct evidence of the withdrawal of fluoride from bone has been obtained in cattle (*6*) and in rats (*7–9*).

It was not ascertained by these studies whether or not any factors in association with a reduction in fluoride intake influenced the mobilization of fluoride. It may be surmised, however, that variations in the metabolic activity of skeletal tissue due to age could alter the process. In support of this assumption there is extensive evidence that the degree of skeletal maturation affects the deposition and retention of ions other than fluoride normally present in calcified tissues (*10*).

The purpose of the present study was to investigate the relationship of age to the rate of mobilization of fluoride in a human population group exposed to an excessive amount (8 p.p.m.) of waterborne fluoride.

ORGANIZATION OF THE STUDY

For more than 50 years before defluoridation was begun on March 10, 1952 (*11*), the communal water supply of Bartlett, Tex., contained 8 p.p.m. fluoride. Since this date the fluoride in the water has been maintained at approximately 1 p.p.m.

One hundred and sixteen white males who had used the Bartlett water supply for at least 2 years immediately prior to defluoridation and who were currently drinking Bartlett water composed the study population. Their numerical distribution according to age group, together with data relative to duration of exposure to the untreated water, is shown in table 1.

Age group (years)	Number of persons	Number of years of exposure		
		Maximum	Minimum	Mean
7	9	7	7	7
8	7	8	2	6
9	6	9	2	6
10	9	10	4	7
11	4	11	4	9
12	5	12	6	10
13	7	13	2	9
14	3	14	5	11
15	9	15	9	13
16	6	16	7	12
20–29	8	20	5	12
30–39	7	28	4	11
40–49	13	47	8	26
50–59	7	57	4	32
60–69	7	50	4	30
70 and over	9	50	4	34

Table 1.—*Age distribution of the study group and exposure to 8 p.p.m. fluoride in drinking water*

On the day defluoridation was begun (which was, of course, before the defluoridation process had changed the fluoride content of the water supply) and at intervals thereafter during the next 113 weeks, a spot urine specimen was obtained from each person in the study. Aliquots of the specimens were pooled by age groups and analyzed for fluoride by standard procedures (*12, 13*), with calcium hydroxide (1 gm. per 100 ml. urine) employed as the fluoride fixative.

RESULTS

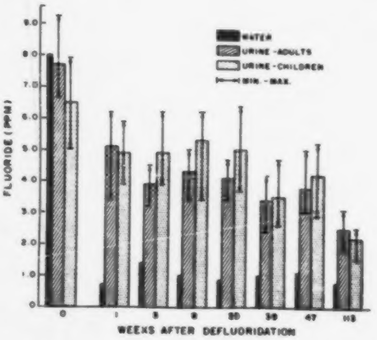
The mean daily fluoride content of the treated water in Bartlett during the period of observation was 1.32 p.p.m. The average fluoride content for each month is given in table 2.

The urinary fluoride values for each age group appear in table 3, and the means for all children, ages 7 through 16, and all adults, ages 20 and over, are shown in the chart. This division of the study population into children and adults was based on the fact that skeletal maturation is complete by approximately 18 years of age (*14*).

On the day defluoridation was begun, the fluoride content of the urine samples ranged from 5.0 to 9.2 p.p.m. and averaged 6.5 p.p.m. in children and 7.7 p.p.m. in adults. One week later, the water contained 0.7 p.p.m. fluoride, and the mean concentration of urinary fluoride had decreased to 4.9 p.p.m. in children and 5.1 p.p.m. in adults. After 5 weeks the mean urinary fluoride concentration remained unchanged in children but had decreased to 3.9 p.p.m. in adults. No further changes were apparent at the end of 9 weeks and 20 weeks, but the fluoride content of the urine had decreased in both children and adults after 39 weeks. After 113 weeks, the average concentration of fluoride in the urine was 2.2 p.p.m. in children and 2.5 p.p.m. in adults.

DISCUSSION

It has been shown that persons whose drinking water contained 0.5 to 5.1 p.p.m. fluoride excreted fluoride in the urine approximately equal in concentration to that in the drinking water (*15*). On the basis of this evidence, the urine of the Bartlett residents would be expected to contain 1.0–1.5 p.p.m. of fluoride following defluoridation of their drinking water. The fact that the urinary concentrations of fluoride considerably exceeded these expected values agrees with previous reports that urinary excretion of fluoride continued to be high after a marked reduction in the fluoride intake. It also indicates that fluoride is being mobilized from the skeletal tissues of the Bartlett residents.



Fluoride content of water and urine at specified intervals after defluoridation of the water supply in Bartlett, Tex.

There was no apparent correlation of age with urinary excretion of fluoride within either the group 7 through 16 years of age or the group aged 20 years and over. When the mean fluoride values for these two groups are compared, however, it will be noted that during the first 20 weeks the urinary excretion of fluoride decreased somewhat less precipitously in children than in adults. This finding suggests that the degree of mobilization of skeletal fluorine was somewhat greater in children than in adults during this period.

It has been suggested that the withdrawal of fluoride from bone can be attributed to (*a*) exchange phenomena in which fluoride ions on the surface of the apatite crystals exchange with hydroxyl ions in the extracellular fluids and (*b*) to the basic resorption-deposition cycle of the skeleton (*16*). Since this latter process is identified with bone growth and reconstruction, it seems probable that exchange is the principal mechanism concerned in the mobilization of fluoride from the mature skeleton. On this basis the initial rapid decrease in the urinary concentration of fluoride in adults presumably reflects the loss of readily exchangeable, surface-bound fluoride, whereas the somewhat less precipitous drop in children may be the consequence of the progressive liberation of fluoride from bone through resorptive activity.

SUMMARY

The urinary excretion of fluoride was determined in children, ages 7–16 years, and in adults, ages 20 years and older, following the reduction of fluoride in their drinking water from 8 p.p.m. to approximately 1 p.p.m. During a period of 27 months, the concentration of fluoride in urine specimens decreased from 6–8 p.p.m. to approximately 2 p.p.m. The urinary fluoride values during the period were considerably higher than would be expected for a corresponding group with no prior exposure to high levels of fluoride. These values indicate that previously stored fluoride was being mobilized. There was no apparent relation between age and urinary fluoride excretion within either group. However, the extent of mobilization appeared to be greater in children than in adults.

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Table 2.—*Average monthly concentrations of fluoride (p.p.m.) in the water supply of Bartlett, Tex., after defluoridation*

Year	January	February	March	April	May	June	July	August	September	October	November	December
1952	-------	-------	1. 75	0. 98	1. 08	1. 28	1. 50	1. 46	1. 30	1. 74	1. 41	1. 23
1953	1. 13	1. 08	1. 26	1. 17	1. 09	1. 39	1. 48	2. 13	1. 27	1. 14	1. 30	1. 36
1954	1. 16	1. 43	1. 24	1. 25	1. 06	-------	-------	-------	-------	-------	-------	-------

Table 3.—*Fluoride content of urine (in p.p.m.) for specified age groups after defluoridation of drinking water*

Age group (years)	Number of weeks after defluoridation							
	0 (Mar. 10, 1952)	1 (Mar. 17, 1952)	5 (Apr. 14, 1952)	9 (May 15, 1952)	20 (July 25, 1952)	39 (Dec. 9, 1952)	47 (Feb. 4, 1953)	113 (May 11, 1954)
7	6. 9	3. 9	4. 6	5. 3	3. 9	3. 9	4. 0	2. 5
8	5. 5	5. 0	4. 9	6. 2	5. 6	2. 6	4. 8	2. 2
9	5. 6	4. 5	3. 9	3. 4	4. 3	2. 9	3. 0	2. 2
10	6. 1	4. 3	4. 2	4. 9	4. 8	3. 5	4. 0	2. 1
11	7. 2	5. 1	5. 0	5. 4	6. 4	3. 0	4. 2	2. 4
12	7. 9	5. 1	5. 0	6. 0	4. 8	3. 5	3. 9	2. 1
13	6. 1	5. 2	5. 5	5. 7	5. 3	4. 7	5. 2	2. 0
14	7. 7	4. 8	6. 2	5. 2	3. 7	4. 0	5. 0	1. 5
15	7. 1	5. 9	4. 8	5. 5	5. 3	3. 2	3. 8	2. 5
16	5. 0	4. 7	4. 7	5. 6	5. 7	3. 8	3. 7	2. 1
Average	**6. 5**	**4. 9**	**4. 9**	**5. 3**	**5. 0**	**3. 5**	**4. 2**	**2. 2**
20–29	6. 8	3. 4	3. 8	4. 1	4. 0	3. 6	3. 5	2. 7
30–39	8. 2	4. 4	3. 3	3. 4	3. 7	3. 2	3. 4	1. 8
40–49	9. 2	5. 7	4. 5	5. 0	4. 7	4. 2	-------	2. 1
50–59	6. 6	5. 3	4. 4	4. 4	4. 2	3. 8	5. 0	2. 6
60–69	8. 5	5. 4	4. 1	4. 7	3. 4	2. 4	3. 9	2. 6
70 and over	7. 0	6. 2	3. 2	3. 9	4. 5	3. 4	3. 0	3. 1
Average	**7. 7**	**5. 1**	**3. 9**	**4. 3**	**4. 1**	**3. 4**	**3. 8**	**2. 5**

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URINARY FLUORIDE LEVELS ASSOCIATED WITH USE OF FLUORIDATED WATERS

By I. ZIPKIN, R. C. LIKINS, F. J. McCLURE, and A. C. STEERE

Previous studies have demonstrated a close correlation between the concentration of fluoride in the urine and the fluoride occurring naturally in drinking water (*1, 2*). Only a limited quantity of data is available, however, concerning the urinary excretion of fluoride added to a municipal water supply (*2–4*). Moreover, it was not determined in any of these studies how soon after beginning the regular ingestion of a fluoride water a stabilized equilibrium is attained, nor was any attempt made to investigate the rate of fluoride excretion in individuals of various ages.

The present study was undertaken, therefore, to provide the following information with respect to fluoridated drinking waters: (*a*) the relation between the level of fluoride in the drinking water and the concentration of fluoride in the urine, (*b*) the time required after fluoridation for this relation to become stabilized, and (*c*) the effect of the age of the individual on the time required for establishment of this equilibrium.

The advent of water fluoridation in Montgomery County, Md. in December 1951 made it possible to obtain such information. Additional, preliminary information was available from Grand Rapids, Mich., where the urinary excretion of fluoride had been under observation since the start of water fluoridation in January 1945. For comparison with the data from these two areas, the results of a survey in 1948 in Aurora, Ill., are presented. The drinking water in Aurora has contained approximately 1.0 p.p.m. fluoride from natural sources since 1895 (*2*).

SPOT SPECIMENS VS. 24-HOUR SAMPLES

The difficulties in obtaining 24-hour urine specimens in a study of this extent are obvious. Accordingly, spot specimens from a number of individuals were pooled in equal volumes to provide a sample for fluoride analysis. Early in the study, information was obtained regarding the relation of the fluoride content of spot specimens to that of the 24-hour total volume.

During a 24-hour collection period, spot specimens were taken between 9 and 12 a.m., 2 and 5 p.m., and 8 and 11 p.m., from 9 adult males who were drinking water containing 1.0 p.p.m. fluoride provided as sodium fluosilicate. These samples, as were all samples in the study, were alkalized with calcium oxide prior to evaporation and ashing and analyzed for fluoride according to standard procedures (*5, 6*). The results are shown in table 1.

The fluoride concentration in a single spot specimen may vary considerably

TABLE 1.—*Fluoride concentration (p.p.m.) in spot and 24-hour urine specimens, Montgomery County, Md.*

Subject	Spot specimens			Mean of spot specimens	24-hour specimen [1]
	9–12 a. m.	2–5 p. m.	8–11 p. m.		
A	0.3	0.5	0.5	0.4	0.6
B	.4	.5	.5	.5	.5
C	1.5	1.1	1.3	1.3	1.1
D	1.2	1.3	.8	1.1	1.0
E	.8	.9	.8	.8	.8
F	.6	1.5	2.0	1.4	1.2
G	1.5	.8	1.5	1.2	1.2
H	.8	.9	.9	.9	.6
I	1.1	1.5	1.4	1.3	.9
Mean	.9	1.0	1.1	1.0	.9

[1] Corrected for spot specimen analysis.

from that in the 24-hour sample. However, according to the *t* test, the mean concentrations for the 3 spot specimens from the 9 men (0.9 p.p.m., 1.0 p.p.m., and 1.1 p.p.m.) did not vary significantly from the mean fluoride concentration of 0.9 p.p.m. for the 24-hour samples.

These results apply to exposure to 1.0 p.p.m. fluoride in the drinking water. They agree very well with the data obtained by Largent and Ferneau for other levels of fluoride (7). It appears, therefore, that the fluoride concentration of pooled urine specimens for a group of individuals reflects reasonably well the average fluoride concentration in 24-hour specimens.

FLUORIDATION OF THE DRINKING WATER

Beginning December 28, 1951, the drinking water of Montgomery County, Md., has been fluoridated to approximately 1.0 p.p.m. fluoride by the addition of sodium fluosilicate. The water supplying this area is obtained from the northwest branch of the Anacostia River (*8*). Water samples taken at widely scattered points in the study area for 10-day periods at the time of the urine collections showed approximately 1.0 p.p.m. fluoride after an initial adjustment period lasting 2 to 4 weeks.

The communal water of Grand Rapids, Mich., which is obtained from Lake Michigan, has been fluoridated for more than 10 years with sodium fluoride. The fluoride content has been maintained at a relatively constant level of 1.0–1.1 p.p.m. (*9*).

SAMPLING PROCEDURES

The subjects studied in Montgomery County were male school children and male adults who had had no exposure to a fluoride-containing drinking water before the start of fluoridation and who were in continuous residence thereafter. Nine age categories, as shown in table 2, were represented. At the start of the study, the subjects in each age category were divided into four groups according to the time of day the specimen was obtained. The same subjects were followed throughout the course of the study. For the age category 5–14 years, the 4 collection periods were 8:30 to 10:30 a.m.; 10:30 a.m. to 1 p.m.; 1 to 2 p.m.; and 2 to 3 p.m. For the age categories 30–34 and 35–39, the first 2 collection periods were the same as for the younger age groups, and the second 2 were: 1 to 3 p.m. and 3 to 5 p.m.

Eleven collections of urine were made during a period of about 3½ years. For the first 10 collections, the subjects remained in their particular group relative to age and time of day. Approximately 10 specimens, 1 from each subject, were obtained for each of the 36 age-time groups. Equal volumes from these 10 specimens were pooled to provide the sample for analysis. No consistent variations in fluoride content with the time of the day the specimen was taken were observed; therefore, only mean fluoride values for each age category are given in this report. In the 11th collection, the specimens were obtained without regard for the time of day and were pooled for each age category to furnish four samples for analysis.

In the Grand Rapids survey, spot urine specimens were obtained from male school children aged 6 through 17 years. Approximately 15 spot specimens comprised each pool, and from 1 to 4 pools were analyzed for each age group. Continuous residents furnished the specimens for the last three collection periods. This survey, which was begun before the Montgomery County survey, did not parallel it in all details.

FLUORIDE CONCENTRATIONS IN URINE

The data on urinary fluoride concentrations for the Montgomery County residents are shown in table 2. Prior to fluoridation, the fluoride content of the specimens varied from 0.2 to 0.3 p.p.m. As early as 1 week after fluoridation, it was evident that the children were responding quite differently from the adults. At the end of 1 week, the adults' specimens contained 0.7 to 0.8 p.p.m. fluoride. At the end of 6 weeks, they contained the expected 1.0 p.p.m. fluoride (*10*). For these same periods, the fluoride concentration in the urine specimens from the children averaged about half these concentrations, and, although the children varied in age from 5 to 14 years, the fluoride content was essentially uniform for all. As shown by the data for succeeding sampling periods, about 3 years elapsed before the specimens of the children reached 0.9 to 1.1 p.p.m. fluoride.

In table 3, the ratio of urinary fluoride concentration to water fluoride concentration in Montgomery County for each collection period following fluoridation is shown. These ratios illustrate the particularly striking difference between

TABLE 2.—*Fluoride concentration (p.p.m.) in urine specimens, Montgomery County, Md.*[1]

Time after fluoridation	Date	Age, in years										
		5	6	7	8	12	13	14	Mean for 5–14	30–34	35–39	Mean for 30–39
0 week	Dec. 15–29, 1951	0.3	0.2	0.3	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.3
1 week	Jan. 3–5, 1952	.3	.3	.5	.4	.2	.3	.4	.3	.8	.7	.8
2 weeks	Jan. 9–11, 1952	.5	.5	.4	.5	.6	.6	.5	.5	.9	.9	.9
4 weeks	Jan. 23–25, 1952	.4	.4	.5	.6	.6	.6	.6	.5	.9	.9	.9
6 weeks	Feb. 7–11, 1952	.5	.5	.6	.6	.5	.5	.6	.5	1.0	1.0	1.0
10 weeks	Mar. 6–13, 1952	.5	.5	.6	.5	.7	.6	.5	.6	1.0	1.0	1.0
20 weeks	May 14–23, 1952	.7	.6	.5	.6	.6	.6	.7	.6	1.0	.8	.9
1 year, 17 weeks	Apr. 23–28, 1953	.8	.8	.8	.8	.8	.8	1.0	.8	1.2	1.2	1.2
1 year, 49 weeks	Dec. 2–4, 1953	.7	.7	.8	.7	.8	.8	.8	.8	1.1	1.1	1.1
2 years, 48 weeks	Nov. 29–30, 1954	1.1	1.0	1.0	1.1	.9	1.0	1.0	1.0	1.2	.9	1.1
3 years, 24 weeks	May 13–20, 1955	.9	.8	.9	.7	.9	1.0	1.0	.9	1.1	.9	1.0

[1] Fluoridation was begun Dec. 28, 1951.

the children and the adults during the first 2 years (t=8.82, P <0.01). Equilibrium was reached by the adults after 1 week of fluoridation, whereas approximately 3 years was required for equilibrium to be reached by the children.

TABLE 3.—*Ratio of urinary fluoride to water fluoride, Montgomery County, Md.*

Time after fluoridation	Fluoride in water at tap (p.p.m.)	Urinary fluoride/water fluoride ratio, by age group	
		5–14	30–39
1 week	0. 7	0. 4	1. 1
2 weeks	. 8	. 6	1. 1
4 weeks	1. 0	. 5	. 9
6 weeks	. 9	. 6	1. 1
10 weeks	1. 0	. 6	1. 0
20 weeks	1. 0	. 6	. 9
1 year, 17 weeks	1. 0	. 8	1. 2
1 year, 49 weeks	1. 0	. 8	1. 1
2 years, 48 weeks	1. 2	. 8	. 9
3 years, 24 weeks	. 9	1. 0	1. 1

The data on urinary excretion of fluoride for the Grand Rapids residents are shown in table 4. The prefluoridation value of approximately 0.2 p.p.m. fluoride in the urine is consistent with the use of the original Grand Rapids water, which contained 0.1–0.2 p.p.m. fluoride. Approximately 2 months after fluoridation was started, the fluoride content of the urine had increased to 0.6 p.p.m. Approximately 5 years after the start of fluoridation, some of the school pupils were excreting 1.0 p.p.m. fluoride (ages 6, 7, and 17). In the final three collections in Grand Rapids (1952, 1954, and 1955), the excretion of fluoride averaged 1.0 p.p.m. for all age groups. The ratios of urinary fluoride to water fluoride would be the same as the mean urinary fluoride values, since the fluoride content of the water has been kept at a practically constant level of 1 p.p.m.

The 1948 survey in Aurora, Ill., provides the most recent data for that city. The fluoride content of urine specimens from boys aged 8 through 17 years varied from 0.8 to 1.0 p.p.m. and averaged 0.9 p.p.m. All age groups showed approximately the same fluoride concentrations. It remains speculative as to when these subjects arrived at this apparently stabilized relation between fluoride in the urine and fluoride occurring naturally in the drinking water. Similar data pertaining to the urinary excretion of fluoride in Aurora residents are presented elsewhere (*10*).

DISCUSSION

Efficient elimination of fluoride by urinary excretion has been demonstrated in several clinical and experimental studies (*1, 3, 4, 10–12*), and is now considered one of the main deterrents to the accumulation of a toxic amount of fluoride in the body tissues. It has been shown also that fluoride is eliminated with equal facility by persons drinking naturally fluoridated water (*1, 10–12*) and by those drinking artificially fluoridated water (*3, 4*).

The surveys in Montgomery County and Grand Rapids demonstrated a similar high level of efficiency in the urinary elimination of fluoride added to the drinking water as sodium fluosilicate or sodium fluoride. The findings confirm the results of previous studies, which showed that approximately 1.0 p.p.m. fluoride is found in the urine when fluoride is ingested in drinking water containing 1.0 p.p.m. from natural sources (*10*) or added as sodium fluoride (*3, 4*). The findings are regarded as evidence that no hazard of cumulative toxic fluorosis is associated with the use of fluoridated water containing 1.0 p.p.m. fluoride.

An important consideration pertaining to water fluoridation is the time required for a stabilized equilibrium between water fluoride and urinary fluoride to be reached. That the period of adjustment can be remarkably short is clearly shown by the data for the adults in Montgomery County. As early as 1 week after fluoridation, the fluoride concentration of the urine was approximately that of the drinking water.

There is some indication that the period of adjustment was somewhat shorter for the children of Montgomery County than for those of Grand Rapids. The suggested difference in the two areas cannot reasonably be ascribed to a difference in the fluoridating agents, as it has been shown in studies with the white rat that sodium fluoride and sodium fluosilicate have similar metabolic effects (*11, 14*). It is possible that the variation in sampling procedures was at least partly responsible for the seeming difference. It is also possible that the difference was due partly to the quantity of water ingested. Water consumption, and thus total fluoride ingested, may have been higher in Montgomery County, owing to the consistently higher mean monthly temperature in that area than in Grand Rapids. The temperature difference averages about 10 degrees throughout the year, according to information from the United States Weather Bureau. It is significant that in both Montgomery County and Grand Rapids the children reached the same state of apparently stabilized equilibrium between urinary fluoride concentration and water fluoride concentration as the adults in Montgomery County, even though they required a somewhat longer period of time.

Speculation as to the reasons for the apparently different response of the children and the adults must turn largely to the possibility of basic differences in fluoride metabolism reflected in the skeletal retention of fluoride. The rapid

TABLE 4.—*Fluoride concentration (p.p.m.) in urine specimens, Grand Rapids, Mich.*[1]

Time after fluoridation	Date	Age, in years												
		6	7	8	9	10	11	12	13	14	15	16	17	Mean
0 weeks	Jan. 17–18, 1945	----	----	----	----	----	----	0. 1	0. 1	0. 2	0. 1	0. 2	0. 3	0. 2
7 weeks	Mar. 6–8, 1945	0. 6	0. 6	0. 5	0. 7	0. 8	0. 6	. 7	. 7	. 6	. 6	. 7	. 7	. 6
3 years, 18 weeks	May 20–21, 1948	. 7	. 8	. 6	. 7	. 6	. 7	. 6	. 7	. 7	. 8	. 8	. 7	. 7
3 years, 43 weeks	Nov. 15–17, 1948	----	----	. 7	----	. 8	----	. 8	----	. 8	----	. 7	----	. 8
4 years, 43 weeks	Nov. 15–17, 1949	1. 0	1. 0	. 6	. 6	. 8	----	. 8	----	. 6	. 6	. 6	1. 0	. 8
7 years, 37 weeks	Sept. 30–Oct. 7, 1952	. 8	1. 0	. 8	. 9	. 8	1. 0	1. 3	. 9	. 9	1. 1	. 9	1. 1	1. 0
9 years, 17 weeks	May 13–14, 1954	1. 0	. 8	. 8	1. 0	1. 0	1. 0	. 8	. 7	1. 0	1. 0	. 8	. 9	. 9
10 years, 19 weeks	May 25–26, 1955	. 9	. 9	. 9	. 8	1. 0	1. 0	1. 1	1. 1	. 9	. 9	. 9	1. 0	1. 0

[1] Fluoridation was begun Jan. 25, 1945.

attainment of equilibrium in adults suggests certain limitations in the capacity of mature bone to deposit fluoride. In this connection it may be noted that the mature rat, without prior exposure to fluoride, stored considerably less fluoride in bones and teeth than did the young growing rat, although they ingested equal amounts of fluoride in their drinking water (*15*). Decreasing amounts of fluoride were stored by rats as the rate of growth diminished, and at maturity a relatively constant level of skeletal fluoride was attained. On the basis of these experimental data, it would seem reasonable for adults to excrete more fluoride than children and hence reach an apparent equilibrium between the concentration of urinary fluoride and water fluoride at an earlier date. The data of the present study thus lend support to the proposition that the retention of fluoride by bone is influenced by its state of maturity.

The length of time required for the children to reach a relatively constant concentration of urinary fluoride suggests some elevation in the fluoride deposited in the skeletal tissues during this adjustment period. However, this increase in retained fluoride would seem to be very small. The urine during the adjustment period contained 0.5–0.6 p.p.m. fluoride, providing for the elimination of substantial quantities of the waterborne fluoride. It has been shown that no adverse effects on the growth and development of the carpal bones accompany the continuous use of natural fluoride drinking water containing approximately 3.0 p.p.m. or more of fluoride (*16*). In addition, examinations of children after 10 years of use of water supply containing 1.2 p.p.m. fluoride added as sodium fluoride failed to reveal anything unusual with respect to the blood, urine, height, or weight; and roentgenographic studies on the hand, wrist, knees, and lumbar spine of these children showed normal development (*17*). These observations support the conclusion that whatever this increase in the retention of skeletal fluoride by these younger age groups may have been, it was not a health hazard.

SUMMARY

The concentration of fluoride in the urine was determined in individuals of different ages who were drinking water fluoridated to approximately 1.0 p.p.m. fluoride with sodium fluosilicate (Montgomery County, Md.) or with sodium fluoride (Grand Rapids, Mich.).

In adults (Montgomery County), the water fluoride and urinary fluoride concentrations became approximately equal within 1 week after the introduction of the fluoridated water. In school children aged 5 through 14 years (Montgomery County) and 6 through 17 years (Grand Rapids), a considerably longer period of time (approximately 3 and 5 years respectively) elapsed before the concentration of fluoride in the urine reached that in the drinking water.

The difference in the response of adults and children during the initial period of exposure to a fluoridated drinking water suggests that the maturity of human skeletal tissue influences its capacity to retain fluoride.

The results of the study do not suggest any essential difference in urinary elimination of fluoride ingested in naturally fluoridated drinking water and the elimination of fluoride ingested in drinking water fluoridated with either sodium fluoride or sodium fluosilicate.

The findings are regarded as evidence that no hazard of cumulative toxic fluorosis is associated with the use of a drinking water fluoridated to contain 1 p.p.m. fluoride.

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John D. Rust and Frederick A. Bullock, formerly with the laboratory, assisted in collecting the specimens in Montgomery County, Md. Virginia L. DuBois, with the Epidemiology and Biometry Branch of the National Institute of Dental Research, assisted in arrangements for collection of the urine specimens in Grand Rapids, Mich. Dr. Albert L. Russell, chief of that branch, made the statistical analysis of portions of the data.

FLUORIDE DOMESTIC WATER AND PERIODONTAL DISEASE

A. L. Russell

Other reports in this series describe some basic epidemiologic characteristics of periodontal disease in urban (*1*) and in rural (*2*) populations and explore an unknown social factor, indicated by levels of education, which is associated with the severity of disease once present (*3*). This paper summarizes the evidence bearing upon the relation of periodontal disease to use of a fluoride-bearing domestic water, including all pertinent data collected by us through the summer of 1956. The data are based on examinations of nearly 20,000 persons of both sexes, ranging in age from seven to 78 years.

Though many opinions have been expressed in this area, very little epidemioligic evidence has been offered. Brown, Kohli, MacDonald, and McLaren surveyed children aged six to 14 years in three Canadian cities, using the P-M-A index of gingivitis. The domestic waters in these cities carried none, 1.0, and 1.3 p.p.m. of fluorides (F), respectively. The least gingivitis was found in the community with 1.0 p.p.m. of fluoride; rather more gingivitis was found in the fluoride-free community; and substantially more in the community with 1.3 p.p.m. of fluoride (*4*). In 10-year summaries of the fluoridation study at Newburgh, N.Y., Ast and his co-workers have reported that there was slightly but significantly more gingivitis in children of Kingston, the control city, than in children of Newburgh who had used a fluoridated water for from seven to 10 years (*5, 6*). This examination was carried out by the author and its findings will be presented here in greater detail.

METHOD

If any relation between the use of a fluoride domestic water and health or disease in periodontal tissues exists in fact, it should readily be demonstrable by the methods which have established the inhibitory effect of fluoride waters upon dental caries. The most common method has contrasted a fluoride-free population with others using domestic waters with varying fluoride concentrations. Additional evidence has resulted from contrasts of the status of natives in a fluoride community with the status of migrant persons who have used the same water for a shorter period of time. Both methods are applied to periodontal disease in the present report.

The examination and scoring methods previously described (*7*) were used throughout the series. All of the examinations were performed by the author, or by observers directly calibrated with him.

FLUORIDE VERSUS NONFLUORIDE POPULATIONS

Three studies have been carried out by us which contrast the periodontal status of residents of fluoride and nonfluoride communities. They are presented here in order of the ages of the persons examined and of the concentration of fluoride in the three domestic waters.

Newburgh-Kingston — The domestic water of Newburgh, N. Y., was fluoridated early in the summer of 1945 in a study project conducted by Ast and his co-workers, under the auspices of the State of New York Department of Health. The water in nearby Kingston was maintained fluoride-free as a control. Through the courtesy of Ast and his group about 600 children aged seven to 14 years were examined for periodontal disease in each of the cities in March of 1955. The results of this examination are summarized in Table 1.

The proportions of children free from overt signs of disease were higher throughout the age range in children of Newburgh, the fluoride city, than in children of Kingston, the control. For the whole groups the difference between the proportions is significant. (When not stated, the level of significance used is the $P=0.01$ level.) Mean population scores, which reflect both the prevalence and severity of disease, were uniformly higher in Kingston than in Newburgh; for the whole groups the difference is on the borderline of significance at the $P=0.05$ level. This difference is due wholly to the higher prevalence of disease in Kingston. When present, disease was of about equal severity in the two communities. One child with marginal periodontitis which had progressed to the stage of frank pocket formation was found in each city.

Colorado Springs-Boulder — Native adults of Colorado Springs, Colo. (with a domestic water carrying about 2.5 p.p.m. of fluoride) and of Boulder, Colo. (with a domestic water essentially fluoride-free) were examined in the fall of 1950. Their dental caries status has been reported previously (*8*). Data were limited to persons aged 20–44 years because the fluoride content of Boulder water prior to 1906 could not be established. Periodontal findings for these people are summarized in Table 2.

Periodontal disease was more prevalent in lifetime residents of Boulder, using a fluoride-free water, than in lifetime residents of Colorado Springs, who had always used a water containing about 2.5 p.p.m. of fluoride. There were significantly fewer Boulder natives free of disease and significantly more Boulder natives with advanced disease. These differences are reflected in the relative scores. Once initiated, disease was equally severe in the two communities; mean scores for the 89 persons in Boulder

Table 1.—*Relative prevalence and severity of gingival disease in children of Newburgh and of Kingston, N.Y. (Mar. 1955)*

Age Group	Mean Age, Years	No. Examined	Per cent Negative	Mean Score
		Newburgh (1.0 ppm F Since 1945)		
7–9	8.9	238	91.6	0.02
10–12	11.3	352	92.0	0.03
13–14	13.6	29	96.6	0.03
All ages	10.5	619	92.1	0.02
		Kingston (Fluoride-Free)		
7–9	9.0	246	84.1	0.03
10–12	11.3	328	84.8	0.04
13–14	13.6	18	88.9	0.08
All ages	10.4	592	84.6	0.04

TABLE 2.—*Relative prevalence and severity of periodontal disease in native residents of Boulder and of Colorado Springs, Colo. (1950)*

Age Group	Mean Age	Number Examined	Per cent Negative	Per cent with Pockets	Mean Score	Additional Per cent Edentulous *
Colorado Springs (2.5 ppm F)						
20–24	22.7	72	58.3	4.2	0.33	0.0
25–29	27.5	101	55.4	4.0	0.39	0.0
30–34	32.2	82	54.9	15.9	0.66	0.0
35–44	39.6	124	46.0	23.4	0.91	4.6
All ages	31.6	379	52.8	12.9	0.60	1.6
Boulder (Fluoride-Free)						
20–24	22.4	51	52.9	7.8	0.47	0.0
25–29	27.1	41	29.3	19.5	0.81	0.0
30–34	32.0	27	44.4	22.2	0.66	6.9
35–44	39.3	25	16.0	56.0	1.54	26.5
All ages	28.5	144	38.2	22.2	0.79	7.1

* Not included in the totals listed under "numbers examined."

and for the 179 persons in Colorado Springs who exhibited signs of disease were identical at 1.28. As in the Newburgh-Kingston study, the observed differences were wholly due to the higher prevalence of disease in the community using the fluoride-free water.

These findings are based upon direct observation by the examiner. While weaker as evidence, indirect findings based upon histories supplied by examinees were consistent with the direct findings. On the basis of these histories, slightly more teeth per person had been extracted because of periodontal disease in Boulder than in Colorado Springs (*8*). Among persons wholly edentulous three in Colorado Springs (0.8 percent of the population examined) and four in Boulder (2.8 percent of the population examined) testified that periodontal disease had been the major reason why their teeth had been extracted.

Bartlett-Cameron—Smaller groups of older adults were examined in 1953 in Bartlett and in Cameron, Tex. Until a defluoridation plant was installed a short time before this examination Bartlett's domestic water had contained about 8.0 p.p.m. of fluoride. The fluoride content of Cameron's water was about 0.4 p.p.m.

The objective in 1953 was the reexamination of a group of people who had received detailed physical examinations 10 years previously. Of these original subjects, 76 were reexamined in Bartlett and 80 in Cameron. Very few were natives of either city. The average length of residence had been 37 years in Bartlett, and 38 years in Cameron. No differences in periodontal disease, on the basis of clinical and x-ray examination, had been seen in these people in 1943 (*9*). In 1953 there were no significant differences between the groups, as estimated by the P-M-A index and by the degree of alveolar bone resorption disclosed by x-ray (*10*). The relative status of these two groups, as scored by the periodontal index, is shown in Table 3. Because of the small numbers of dentulous persons and the wide age range (25 through 78 years) findings are shown for the total groups.

Nearly all the persons seen in both cities exhibited some sign of disease. Differences seen were small and favored the Bartlett group, which had used the water containing about 8.0 p.p.m. of fluoride. Unlike the two contrasts previously cited there was a difference in severity of disease; periodontal destruction was appreciably more advanced in the low-fluoride group. There were about twice as many persons in Cameron as in Bartlett (11, or 13.8 percent of the group in Cameron against 5, or 6.6 percent of the group in Bartlett) in whom tissue destruction was so advanced that full-mouth extractions were indicated.

Indirect evidence was opposite in direction. About twice as many of the edentulous persons in Bartlett as in Cameron (21, or 26.6 percent of the whole group in Bartlett against 9, or 11.3 percent of the whole group in Cameron) believed that periodontal disease had been the primary reason for loss of their teeth. If the numbers requiring full-mouth extractions are combined with these, tooth mortality findings differ only by chance between the two groups, and group scores for the remaining persons are about the same. This supports the previous conclusion, based on other methods of estimation, that "the periodontal health of the Bartlett and Cameron subjects was approximately the same" (*10*).

Summary—In each of these contrasts the more favorable condition of the periodontal tissues was observed in the fluoride community. In the two adult groups indirect evidence was opposite in direction. The three populations included persons aged seven through 78 years. Two of the groups had used a domestic water containing fluorides considered to be excessive in amount—2.5 and 8.0 p.p.m., respectively. The fluoride water had been consumed for from seven years in children to as long as 44 years in adults. If use of a fluoride domestic water has any adverse effect upon the health of periodontal tissues, it is quite likely that this effect would have been revealed clearly in one or all of these studies. The fact that no adverse effect is evident is wholly incompatible with any hypothesis that use of a fluoride-bearing domestic water is harmful to the periodontal tissues of children or of adults.

On the other hand, the slight differences in favor of the fluoride communities are too weak for acceptance as evidence that use of a fluoride water is beneficial to periodontal tissues. With the exception of Boulder the findings for these groups are very similar in all details to those for the total population of white urban residents so far examined (*1*). In this total population most of the other persons aged 20 years or older were residents of Baltimore, using a fluoride-free water at the time of examination.

TABLE 3.—*Relative prevalence and severity of periodontal disease in residents of Bartlett, Tex. (with an average of 37 years' use of a domestic water containing about 8.0 p.p.m. F) and of Cameron, Tex. (with an average of 38 years' use of a domestic water containing about 0.4 p.p.m. F)*

City	No. Examined	Mean Age	Per cent Negative	Per cent with Pockets	Mean Score	Additional Per cent Edentulous *
Bartlett	45	54.3	4.4	62.2	1.70	40.8
Cameron	64	53.9	3.1	68.8	2.01	20.0

* Not included in the totals listed under "number examined."

Table 4.—*Periodontal scores of native and migrant children in a series of communities with domestic waters containing about 1.0 parts per million of fluorides, 1952–1956*

Group	Year of Examination	Years F Usage, Natives	Natives No.	Natives Mean Age	Natives Mean Score	Migrants No.	Migrants Mean Age	Migrants Mean Score	Difference (Native Score Minus Migrant)
Montgomery Co., Md. (A)	1952	0.0	307	8.2	0.01	195	8.6	0.01	....
	1956	4.0	497	8.3	0.02	443	8.5	0.02	....
Montgomery Co., Md. (B)	1952	0.0	237	13.8	0.06	233	13.9	0.04	+0.02
	1953	1.0	250	13.9	0.19	230	13.6	0.16	+0.03
	1954	2.0	233	13.7	0.10	244	13.7	0.08	+0.02
	1955	3.0	221	13.4	0.04	186	13.5	0.04	....
	1956	4.0	176	13.5	0.05	197	13.5	0.06	−0.01
Prince Georges Co., Md. (C)	1952	0.1	874	9.1	0.03	435	8.9	0.02	+0.01
	1956	4.1	778	8.8	0.03	680	8.9	0.02	+0.01
Prince Georges Co., Md. (D)	1952	0.1	303	13.9	0.06	171	13.8	0.04	+0.02
	1953	1.1	201	14.0	0.21	161	14.1	0.17	+0.04
	1954	2.1	238	13.6	0.05	198	13.7	0.07	−0.02
	1955	3.1	322	13.6	0.08	306	13.6	0.06	+0.02
	1956	4.1	499	13.6	0.11	414	13.7	0.08	+0.03
Prince Georges Co., Md. (E)	1953	1.2	449	14.0	0.36	173	14.1	0.29	+0.07
	1954	2.2	441	13.9	0.26	191	13.9	0.26	....
	1955	3.2	315	14.1	0.24	143	14.2	0.19	+0.05
	1956	4.2	423	13.9	0.18	191	13.9	0.18	....
Muskegon, Mich. (F)	1952	1.3	147	15.5	0.06	95	15.4	0.11	−0.05
	1953	2.3	158	14.1	0.17	96	14.7	0.14	+0.03
	1954	3.3	155	15.1	0.04	106	15.8	0.05	−0.01
Prince Georges Co., Md. (G)	1954	2.3	495	16.9	0.30	258	16.9	0.26	+0.04
Muskegon, Mich. (H)	1955	4.3	645	9.5	0.04	205	10.3	0.08	−0.04*
Grand Rapids, Mich. white (I)	1952	7.8	288	14.4	0.07	147	14.8	0.05	+0.02
	1953	8.8	296	13.7	0.10	139	14.2	0.13	−0.03
	1954	9.8	595	14.5	0.05	431	14.7	0.06	−0.01
	1955	10.8	627	14.4	0.07	501	14.7	0.09	−0.02
Grand Rapids, Mich. Negro (J)	1952–1955	8–11	110	14.6	0.14	279	14.8	0.11	+0.03
Aurora, Ill. (K)	1953	13.5	360	13.5	0.09	207	13.5	0.11	−0.02

* Difference significant at the P = 0.01 level.

NATIVES VERSUS MIGRANTS IN FLUORIDE COMMUNITIES

Marked differences in caries inhibition have been demonstrated by within-group analyses of residents of fluoride areas. Those children who had used the fluoride water since birth have shown significantly lower caries scores than those children who had moved into the community at some time after birth and had, hence, used the same water for a shorter period of time (*11*). Accordingly, a similar analysis was applied to the periodontal data gathered by us in such communities. The examinations of about 18,000 children in communities with domestic water supplies that had contained about 1.0 p.p.m. of fluorides for varying lengths of time are summarized in table 4.

If use of a fluoride domestic water is actually harmful to periodontal tissues, the data of table 4 should reveal a trend opposite in direction to that seen in the inhibition of dental caries, i.e., the longer the fluoride water has been in use by natives, the more migrant children should excel native children in periodontal health. When the duration of fluoride consumption by natives is plotted against the difference in scores between native and migrant children the result is as shown in Figure 1. In this array periodontal scores are generally elevated for a group examined coincident with the peak, or early on the declining slope, of an outbreak of an upper respiratory infection, sometimes influenza A or B (reference 1). This was generally the case in the schools examined in 1953, and was observed sporadically in other years. Comparisons between total scores are misleading unless corrections for this factor are made. But differences between scores within groups are virtually independent of this factor, since the outbreaks tend to affect native and migrant children to an equivalent degree.

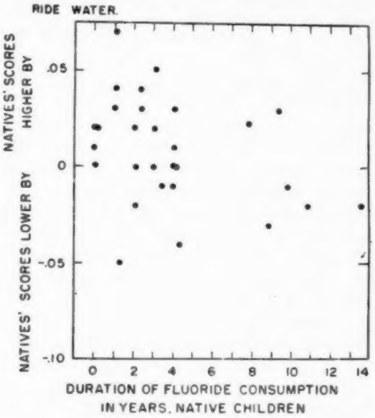

Figure 1.—*Relation between absolute differences in periodontal scores, native and migrant children in fluoride communities, and the number of years native children had consumed a fluoride water.*

Simple inspection of this figure shows that no adverse trend, i.e., a worsening of the relative periodontal status of native children related to the length of time they have consumed a fluoride-bearing domestic water, is apparent. On analysis there is a weak but definite negative correlation indicating that, in general, the greater their advantage in the consumption of water-borne fluoride, the more native children tend to excel migrant children in the health of their periodontal tissues.

As in the fluoride-nonfluoride contrasts, this finding cannot be accepted as evidence that use of a fluoride domestic water enhances the health of periodontal tissues. The difference between a random pattern and that shown in Figure 1 is not great. But the finding is clearly incompatible with any hypothesis that use of a fluoride domestic water is injurious to periodontal health.

This result cannot be ascribed to examiner bias, since the residence history of none of the children was known to the observer at the time of examination.

SUMMARY AND CONCLUSIONS

The periodontal status of residents of three fluoride communities has been compared with the periodontal status of residents of three comparable low- or nonfluoride communities. Ages of the persons studied ranged from seven to 78 years. The domestic water supplies of the fluoride communities carried about 1.0, about 2.5, and about 8.0 p.p.m. of fluorides, respectively. Fluoride subjects had used the domestic water for from seven to 44 years. Indirect evidence in the two adult groups was opposite in direction. In each case the fluoride group exhibited the more favorable periodontal condition on direct examination.

Native school children in a series of communities, each with about 1.0 p.p.m. of fluoride in its domestic water, were compared with migrant children in the same communities. There was a weak tendency for the periodontal health of native children to improve, relative to that of migrants, as their advantage in water-borne fluoride consumption increased.

Most of the differences were slight. They are not adequate to support a hypothesis that use of a fluoride water results in improved health of periodontal tissues. The findings are, however, wholly incompatible with any hypothesis that the periodontal tissues of children or of adults are harmed by use of a fluoride-bearing domestic water.

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RATE OF URINARY FLUORIDE OUTPUT IN NORMAL ADULTS

I. Zipkin (with the technical assistance of W. A. Lee) and N. C. Leone

Previous investigations have emphasized the importance of the kidney in the elimination of fluoride from the human body (*1–5*). In earlier fluoride balance studies (*4–5*) the urinary output of fluoride was determined over relatively long periods of time. However, it was felt that the rate of urinary fluoride output could be better assessed over short intervals of time. In the present study the hourly rate of urinary fluoride elimination was determined in normal adults: (*a*) drinking water containing 1 p.p.m. F, and (b) receiving in addition a "challenge" dose of 5 mg F as NaF.

EXPERIMENTAL

Eight normal adults 26–58 years of age, drinking a municipal water containing 1 p.p.m. F, served as subjects for the present investigation. At 9:00 a.m. of the control day the bladder was emptied. Two hundred ml of tap water was drunk to insure an adequate urinary output and the urine voided during the next hour was collected. This procedure was repeated at 10:00 a.m., 11:00 a.m., 12:00 noon, and at 2, 4, 6, 8, and 10 p.m. Two hundred ml of tap water was also drunk at 12 p.m. and the urine voided between 12 midnight and 9:00 a.m. of the next day was collected as a single sample. Next, 200 ml water containing an additional 5 mg F as NaF was drunk; the regimen of the previous control day was repeated; and the study was completed at 9:00 a.m. of the day following the test day. Thus, three hourly urine samples, six 2-hour samples, and one overnight sample of 9 hours were obtained from each of the eight subjects during the control and test day. The subjects refrained from tea and fish, because of the high fluoride content, and recorded the kind, time, and amount of all ad libitum fluid consumption. All fluids exclusive of drinking water were assumed to contain 0.2 p.p.m. F, and the daily diet was estimated to contain 0.3 mg F (*6*).

Analytical Procedure—For analysis of fluoride, 1 gm of low-fluoride CaO, prepared according to reference No. 7, was added to a volume of urine estimated to contain approximately 100 μg of fluoride, and the sample was evaporated to dryness in a platinum dish on a steam bath. The samples were dried overnight at 105° C and ashed slowly as follows: the urines were placed in a cold muffle furnace set at 600° F, removed after 40 minutes of heating, thoroughly triturated with wa-

Table 1.—*Fluid intake and urinary output during a 24-hour period of eight normal adults drinking water containing 1 p.p.m. F and receiving a supplement of 5 mg F as NaF*

		Intake ml	Output ml	Per cent Intake Excreted
1 ppm F	Range	2,000–5,400	1,356–4,181	46.2–108.6
	Mean	3,089	2,600	84.9
1 ppm F + 5 mg F as NaF	Range	2,200–4,825	891–4,035	40.5–109.7
	Mean	3,048	2,448	79.3

Table 3.—*Percent of total fluoride intake excreted in the urine in 24 hours (comparison with values in literature)*

	F Intake mg	F Output mg	Per cent Intake Excreted
Zipkin and Leone	3.2	2.1	65.1
	8.1	4.4	54.1
Mean	5.7	3.3	59.6
McClure, et al.[4]	4.7	2.9	61.6
	5.2	3.6	69.3
	5.5	4.3	78.2
	6.2	3.8	61.2
	6.8	4.2	61.7
	7.2	4.4	61.1
	7.4	4.4	59.5
	8.9	5.3	59.6
	9.5	4.4	46.3
Mean	6.8	4.2	61.9

ter, evaporated to dryness, and dried overnight at 105° C. This ashing regimen was repeated at 800° F for 20 minutes and twice at 1,020° F for 45 minutes. The samples were then analyzed for fluoride by established procedures (*8, 9*).

RESULTS AND DISCUSSION

The volumes of fluid intake and urinary output are shown in table 1. No statistically significant differences (*10*) were found in the per cent of the fluid intake eliminated by the kidney during the control and test days ($p > 0.05$).

The total amount of fluoride ingested and eliminated by the kidneys during the control and test days is shown in Table 2. It is apparent that the percent of ingested fluoride eliminated by the kidneys during the test day (54.1) was significantly less ($p < 0.01$) than that excreted during the control day (65.1). These data corroborate the earlier work of McClure, et al. (*4*), as shown in table 3.

It should also be noted that in both these studies approximately 60 percent of the total fluoride ingested appeared in the urine. The remainder is found in the perspiration and feces (*4*).

The urinary output of fluoride, expressed on a mg/hr basis for each of the 10 collection periods during the control and test day, is shown in figure 1. During the control day when the subjects ingested water containing 1 p.p.m. F, fluoride was eliminated at a relatively uniform rate of about 0.1 mg/hr. The rate of urinary output of fluoride was at a maximum one hour after ingestion of the "challenge" dose of 5 mg F as NaF and then dropped rapidly during the succeeding eight hours, finally approaching the rate of 0.1 mg/hr found during the control day.

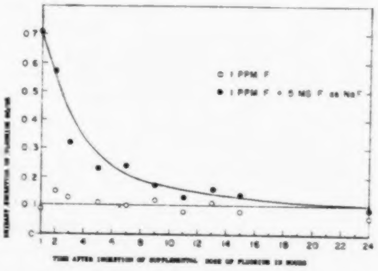

Figure 1.—*Urinary excretion of fluoride (mg/hr) in eight normal adults drinking water containing 1 p.p.m. F and receiving a supplement of 5 mg F as NaF.*

As shown in figure 1, 1.6 mg of fluoride was found in the urine during the first 3 hours after the ingestion of the "challenge" dose of 5 mg of fluoride. This eight normal adults drinking 1 p.p.m. F and receiving a single "challenge" dose of 5 mg F as NaF in 200 ml of water (test day). The total fluoride output after each time interval was compared with that found at a similar time during represented approximately 20 percent of the total fluoride ingested during the test period (table 2). Hodge (*11*), reporting the unpublished work of Smith and Gardner, states that normal young adults drinking water containing 1 p.p.m. F and ingesting an additional single dose of 1.5 mg F, excreted 25 percent of the total fluoride intake in 3 hours. In the present study the highest hourly rate of output of fluoride following ingestion of the "challenge" dose was observed during the first hour.

SUMMARY

Urine samples were collected at 10 intervals during a 24-hour period from the previous 24-hour period (control day) when 1 p.p.m. F alone was ingested in the drinking water.

After 24 hours 65.1 percent of the fluoride ingested during the control day appeared in the urine, whereas only 54.1 percent of the intake during the test day was found.

Fluoride was eliminated with the greatest rapidity during the first hour after ingestion of a "challenge" dose of 5 mg F. Thereafter, the rate dropped rapidly for an additional 8 hours and then approached the rate of 0.1 mg/hr observed during the control day.

ACKNOWLEDGMENT

The assistance of Patricia B. Geiser, Public Health Service senior assistant nurse officer, in the organization and execution of part of the study is gratefully acknowledged. The aid of Jane Soban in performing some of the fluoride analyses is also gratefully acknowledged.

Table 2.—*Fluoride intake and urinary fluoride output during a 24-hour period of eight normal adults drinking water containing 1 p.p.m. F and receiving a supplement of 5 mg F as NaF*

		Intake * mg	Output mg	Per cent Intake Excreted
1 ppm F	Range	2.3–5.5	1.5–3.6	55.6–72.0
	Mean	3.2	2.1	65.1
1 ppm F + 5 mg F as NaF	Range	7.0–10.0	3.3–5.5	47.1–63.2
	Mean	8.1	4.4	54.1

* All fluids exclusive of drinking water were assumed to contain 0.2 ppm F. It was estimated that 0.3 mg F were consumed daily in the diet.

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EFFECTS OF FLUORIDATION ON GENERAL HEALTH AS REFLECTED IN MORTALITY DATA

By Thomas L. Hagan

During the course of the investigations of the relationship between the ingestion of water-borne fluorides and dental fluorosis and dental caries, there was no indication of an association between exposure to low level fluoride intake and any abnormal physical findings exclusive of mottled enamel. Almost concurrently, however, with the first reports validating the hypothesis that the fluoride supplementation of public water supplies reduces dental caries there came the allegation that human mortality was higher in fluoride-water areas than in nonfluoride areas.

It is difficult to assess the basis of rumors of this nature. In view of the fact that there has never been any valid evidence offered to demonstrate that water fluoridation shortened the lifespan of any individual one must assume that the charges stem from misunderstanding or misuse of crude mortality data.

Nonetheless in view of the gravity of the charge concerning the safety of fluoridation, it has been essential to determine whether or not a relationship exists between mortality and the ingestion of water-borne fluorides.

It is the purpose of this paper to describe three studies which test the relationship between exposure to low level fluoride intake and mortality. Manifestly an examination of mortality experience provides only indirect evidence concerning general health effects resulting from the use of fluoridated water.

The first of these studies was reported in the Health Bulletin of the Illinois Health Department and was based on 1940 census and mortality reports (*1*). This analysis compared mortality rates for deaths from all causes and for heart disease, cancer, nephritis, and diabetes among Illinois communities using fluoride-bearing water and communities using fluoride-free water. Data from four Illinois cities, over 10,000 population, using water containing 0.70 p.p.m. fluoride from natural sources were compared with other Illinois cities of like size using fluoride-free water. Based on the data presented, it was concluded that "mortality experience in Illinois offers little or no support for claims of adverse effects being produced by limited ingestion of fluorides."

The second study is concerned with comparisons of mortality between 32 fluoride cities and 32 nonfluoride cities in different parts of the country (*2*). The populations included 892,625 persons in the fluoride cities and 1,297,000 in the nonfluoride cities.

This analysis was set up by listing all of the cities in the United States over 10,000 population in 1950, whose water supplies contained 0.70 p.p.m. fluoride or more from natural sources. The criterion for inclusion of a city was met when the majority of its water anlysis, for the entire period for which fluoride readings were reported, showed at least 0.70 p.p.m. Each fluoride city thereupon was paired with the nearest nonfluoride city over 10,000 population. The criterion for a nonfluoride city was a fluoride content of 0.25 p.p.m. or less according to all available reports of water analyses.

This procedure resulted in an array of 32 pairs of cities, each pair consisting of one city whose water supply contained 0.70 p.p.m. fluoride or more and an adjacent city whose water supply contained 0.25 p.p.m. or less. The 1950 mortality rates of these cities for deaths from all causes, from heart disease, from cancer, or from nephritis showed no discernible differences related to the presence or absence of fluorides in their water supplies.

Table 1 makes a direct comparison between each of the 32 pairs. It shows that heart disease mortality rates were higher in 17 of the nonfluoride cities than those in each paired fluoride city; that for cancer and for deaths from all causes half or 16 of the rates for the nonfluoride cities were higher; and that for nephritis 19 of the nonfluoride cities were higher. It is evident that there is no real difference in mortality experience attributable to fluoride water usage between the 32 fluoride and the 32 nonfluoride cities for these causes of death.

The third analysis of this presentation concerns an examination of mortality experience in five cities before and after controlled water fluoridation was instituted. Each community represents the largest city to begin fluoridation in a given year from 1945 through 1949. Table 2 shows these cities, Grand Rapids, Mich.; Sheboygan and Madison, Wis.; Evanston, Ill.; and Charlotte, N.C., with their 1940 and 1950 populations and the year fluoridation of the respective water supplies was begun. The populations are large enough to give stability to mortality data for purposes of comparison.

In this study, the 1940 and 1950 mortality experiences in each of these cities were compared to determine whether the institution of fluoridation was associated with a change in the number of deaths. Inasmuch as 1940 and 1950 were census years, the population count serves as a firm base for computed mortality (*3*). Data for intercensal years while carefully estimated, lack the precision of true counts necessary for a study of this nature. Since the first controlled fluoridation installation was made in 1945, the comparison made here provides some indication of the effects of 1, 2, 3, 4, and 5 years of fluoride ingestion on mortality.

Table 1.—*Comparison of mortality between pairs of fluoride and nonfluoride cities for selected causes of death*

	Mortality cause			
	All causes	Heart	Cancer	Nephritis
Number of pairs in which mortality was greater in fluoride cities.	16	15	16	13
Numbers of pairs in which mortality was greater in nonfluoride cities.	16	17	16	19

Table 2.—*The largest cities beginning to fluoridate their water supplies during each of the years 1945–49, along with their populations in 1940 and 1950*

City	Year fluoridation started	Population	
		1940	1950
Grand Rapids, Mich	1945	164,292	176,515
Sheboygan, Wis	1946	40,638	42,365
Evanston, Ill	1947	65,389	73,641
Madison, Wis	1948	67,447	96,056
Charlotte, N.C	1949	100,899	134,042

Table 3.—*U.S. mortality rates, all causes, per 100,000 population by age, race and sex (1940 and 1950)*

Age	White		Nonwhite	
	Male	Female	Male	Female
1940				
Under 1	5,670	4,359	10,122	7,737
1–4	281	241	527	443
5–14	112	81	164	144
15–24	197	140	499	503
25–34	281	218	853	740
35–44	507	367	1,318	1,175
45–54	1,139	747	2,453	2,109
55–64	2,522	1,684	3,711	3,318
65–74	5,400	4,154	6,283	5,228
75-84	12,202	10,483	10,877	8,414
85+	25,135	23,495	19,972	15,971
1950				
Under 1	3,401	2,567	5,992	4,749
1–4	136	112	271	230
5–14	67	45	97	75
15–24	152	72	290	216
25–34	185	113	496	390
35–44	381	236	861	754
45–54	985	546	1,857	1,555
55–64	2,304	1,294	3,481	2,763
65–74	4,865	3,243	5,795	4,611
75–84	10,526	8,482	9,030	7,065
85+	22,116	19,680	16,022	13,367

Any comparison of year-to-year mortality in a given community requires that at least two important factors be considered. First, there has been a general decline in mortality for specific causes, notably infectious diseases and neonatal conditions, which is reflected in the total mortality. Table 3 arrays the U.S. mortality rates by age, race, and sex for the years 1940 and 1950 (*4*). The general decline in mortality rates over this 10-year period is apparent. It is clear as well that the characteristics of age, race, and sex affect death rates, with the white female in the most favorable position and the nonwhite male at the greatest risk of death. Two age groups, 5-14 and 45-54, are emphasized graphically to facilitate this comparison.

The second factor is the constant change in the characteristics of a community population occasioned by births, deaths, aging, in-migration, and out-migration. Table 4a gives the populations of five subject cities by race and sex and indicates the extent to which these characteristics of their populations changed between the years 1940 and 1950. Examination of changes in the age characteristics, not shown on this table, indicates that the populations are aging.

Table 4b describes the composition of the several populations in terms of percentages. With respect to changes in the race composition, it should be noted that a 2½ fold increase has occurred in the Grand Rapids nonwhite population and a slight decline in the Charlotte nonwhite group. These examples are cited to point up the fact that to the extent there is a change in population characteristics of the communities, i.e., age, race, and sex, there is to be expected a change in mortality in the community. The availability and utilization of health services resources which reflect themselves in mortality experience changes are more difficult, if not impossible, to equate precisely.

For each of the five cities selected a comparison is made of the "recorded" deaths and the "expected" deaths for each of the census years 1940 and 1950 (table 5). The term "recorded" deaths is that total number of deaths recorded by the community of residence of the deceased. The probability exists that certain long-time residents of the cities

Table 4a.—*Population data for five cities by race and sex (1940 and 1950)*

Place	Year	White		Nonwhite	
		Male	Female	Male	Female
Grand Rapids	1940	78,085	83,482	1,333	1,392
	1950	81,127	88,451	3,433	3,504
Sheboygan	1940	20,115	20,521	1	1
	1950	20,649	21,701	13	2
Evanston	1940	27,067	32,231	2,793	3,298
	1950	30,916	35,591	3,361	3,773
Madison	1940	31,154	35,893	209	191
	1950	46,952	48,171	519	414
Charlotte	1940	33,338	36,137	14,324	17,100
	1950	45,350	51,181	17,538	19,973

Table 4b.—*Population data for five cities. Percent of people in each race and sex category (1940 and 1950)*

Place	Year	White		Nonwhite	
		Male	Female	Male	Female
Grand Rapids	1940	47.53	50.81	0.81	0.85
	1950	45.96	50.11	1.94	1.99
Sheboygan	1940	49.50	50.50	.00	.00
	1950	48.74	51.22	.03	.01
Evanston	1940	41.40	49.29	4.27	5.04
	1950	41.98	48.33	4.57	5.12
Madison	1940	46.19	53.22	.31	.28
	1950	48.88	50.15	.54	.43
Charlotte	1940	33.04	35.81	14.20	16.95
	1950	33.83	38.18	13.09	14.90

Table 5.—*The ratio of recorded deaths to expected deaths in five cities using the U.S. age-race-sex mortality experience as a standard (1940 and 1950)*

City	Year	Number of deaths		Ratio of recorded to expected deaths
		Recorded	Expected	
Grand Rapids	1940	1,783	1,825	0.98
	1950	1,920	1,904	1.01
Sheboygan	1940	431	417	1.03
	1950	415	449	.92
Evanston	1940	665	721	.92
	1950	700	827	.85
Madison	1940	613	670	.91
	1950	715	834	.86
Charlotte	1940	951	870	1.09
	1950	1,081	1,033	1.05

under study would have taken up residence and died elsewhere and conversely the long-time residents of other cities took up residence and died in one of these cities. An assumption must be made that the errors in either direction equalize one another.

The term "expected deaths" is the result of a computation applying the specific age-race-sex mortality rates for the United States as shown previously in figure 3, to the counterpart population cohorts in each of the cities being examined. This procedure is followed in order to facilitate the comparison of each city with itself, using a standard base, in this instance the U.S. mortality experience, for computing an expected mortality value. By this means the recorded deaths may be ratioed against the expected deaths for each city by year. A significant change in this ratio from 1 year to another, 1940 to 1950 for example, would indicate that some variable, other than age, race, sex, and declining mortality which have been equated out, has had some influence.

This chart (table 5) shows the comparison of the number of deaths "recorded" with the number of deaths "expected" for the years 1940 and 1950. The ratios of recorded to expected deaths are shown in the last column. In no instance is there a significant change in the 1950 ratio of recorded deaths to expected deaths from the 1940 ratio. These data therefore do not support the hypothesis or allegation that water fluoridation has caused an increase or a decrease in mortality.

In view of the fact that disease oriented deaths from all causes should be examined, deaths from accidents including motor vehicles, as well as from suicides and homicides, have been excluded in the computations in table 6. A comparison of the ratio of recorded to expected deaths exclusive of violent deaths in the several cities for 1940 and 1950 shows the same pattern as in the examination of total deaths.

In summary, an examination of mortality in fluoride and nonfluoride communities in Illinois, a comparison of mortality in 32 pairs each of one fluoride and one nonfluoride city and an analysis of mortality before and after the institution of a controlled water fluoridation program, do not reveal any relationship between mortality experience and the presence of fluorides in drinking water supplies. These data do not validate the hypothesis that mortality is influenced by the ingestion of water containing fluorides, whether occurring naturally or mechanically added. On the contrary, the nonexistence of a health hazard as demonstrated indirectly by an examination of mortality data, provides another link in the chain of evidence supporting the safety of water fluoridation.

TABLE 6.—*The ratio of recorded deaths to expected deaths in five cities, with deaths due to motor vehicle accidents, other accidents, suicides, and homicides excluded, using the U.S. age-race-sex mortality experience as a standard (1940 and 1950)*

City	Year	Number of deaths		Ratio of recorded to expected deaths
		Recorded	Expected	
Grand Rapids	1940	1,643	1,674	0.98
	1950	1,803	1,764	1.02
Sheboygan	1940	394	380	1.03
	1950	383	416	.92
Evanston	1940	611	660	.93
	1950	672	765	.88
Madison	1940	546	612	.89
	1950	652	762	.86
Charlotte	1940	806	782	1.03
	1950	964	933	1.03

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FLUORIDE CONTENT OF URINARY AND BILIARY TRACT CALCULI

I. ZIPKIN, W. A. LEE and N. C. LEONE

In connection with an extensive survey of the fluoride content of human skeletal tissues (*1*, *2*), it was of interest to assess the relation of water-borne fluoride to the fluoride content of urinary and biliary tract calculi. With the exception of the recent duplicate reports by Herman (*3*) and Spira (*4*), no data are available on the fluoride content of urinary tract calculi. Murray (*5*) reported that kidney stones contained fluoride, but no data were given. Data on the fluoride content of biliary calculi appear to be limited to the recent report of Spira (*6*). In the present study, the fluoride content of urinary and biliary tract calculi was also compared with that of bone obtained from individuals residing in low (0.0–0.6 p.p.m. in the drinking water) and high fluoride areas (2.6 p.p.m. in the drinking water).

Methods and materials.—Thirty-three urinary and 9 biliary tract calculi obtained from individuals with verified histories of fluoride exposure were dried overnight at 105° C. and pulverized. They were then extracted with alcohol for 8 hours and with ether for 4 hours, ashed at 550° C. for 3 hours and analyzed for calcium (*7*), phosphorus (*8*) and fluoride (*9*, *10*, *11*). Samples of dry, fat-free urinary tract calculi were submitted for X-ray diffraction analysis to determine if calculi with strong apatite patterns showed higher concentrations of fluoride. A pooled sample of bile from four individuals who had resided in Washington, D.C. (1 p.p.m. F in the drinking water) was also analyzed for fluoride. The fluoride content of urinary and biliary tract calculi was compared with that of some 35 bones taken at autopsy from individuals residing in low fluoride areas (0.1–0.4 p.p.m.) and in a high fluoride area (2.6 p.p.m. F). Data on the fluoride content of bones from individuals drinking water containing 0.1–0.4 p.p.m. F will be presented elsewhere (*1*).

Results.—The concentration of fat, ash, calcium, phosphorus and fluoride of urinary tract calculi classified according to water-fluoride exposure of the individual is presented in table I.

The mean of 0.25 percent fluoride in urinary tract calculi of individuals from a low fluoride area, expressed on an oven-dry basis, was not significantly different from that of calculi of individuals from an area where the drinking water contained 2.6 p.p.m. fluoride, *i.e.* 0.37 percent. Herman (*3*) and Spira (*4*) reported a range of 0–0.18 percent fluoride with a mean of 0.05 percent fluoride. Their values are considerably lower, in most cases, than those reported for all samples of urinary tract calculi in the present study (0.02–1.11 percent with a mean of 0.31 percent) and may be attributed, to some extent, to the fact that only part of the calculus was used for analysis.

As shown in table I, the Ca/P weight ratio varied widely (0.1–45.7) and was unrelated to the fluoride concentration, Ca/P:F (3.3–762.0). In addition, no relation was apparent between the calcium and fluoride concentrations, Ca/F (35.3–695.0).

The concentration of fat, ash, calcium, phosphorus and fluoride in biliary tract calculi is presented in table II on an oven-dry basis.

As expected, the "fat" comprised at least 90 percent of the calculus in nearly every case. Fluoride was absent, or present in very low concentration. Spira (*6*) also recently reported the presence of small amounts of fluoride in biliary tract calculi similar to those reported in the present study (table II). It is interesting to note that the composite sample of human bile contained only 0.1 p.p.m. fluoride. A sample of ox-bile obtained in this study from an animal exposed to

TABLE I.—*Fat, ash, calcium, phosphorus, and fluoride content of urinary tract calculi* *

Years of residence	Fat	Ash	Ca	P	F	Ca/P	Ca/P:F	Ca/F
	%							
			Low fluoride area†					
1					.41			
4	9.3	63.7	26.1	3.9	.27	6.7	24.8	96.7
9	8.0	66.9	26.[illegible]	4.1	.27	6.5	24.1	98.5
9	.3	67.7			.10			
12	20.1	6.9	13.9	16.0	.02	.9	45.0	695.0
13	11.8	76.8	20.7	15.2	.12	1.4	11.7	172.5
14	8.6	69.9	28.1	3.7	.41	7.6	18.5	68.5
15					.67			
16	20.0	54.1			.06			
20	14.8	71.4	10.7	19.6	.03	.6	20.0	356.7
23					.24			
28	6.5	62.5			.08			
32	6.2	84.6	29.8	15.9	.43	1.9	4.4	69.3
33	17.2	22.4			.12			
33	12.7	58.6	25.8	1.8	.15	14.3	95.3	172.0
51§	4.2	78.0			.66			
			Elevated fluoride area‡					
1	4.0	80.3	28.9	13.2	.82	2.2	2.7	35.3
2	14.6	59.1	23.2	1.8	.16	12.9	80.6	145.0
3					.65			
4§	2.0	80.7	27.3	17.0	.23	1.6	7.0	116.0
9	7.8	63.7	24.5	4.5	.39	5.4	13.8	62.8
13	11.9	68.5	11.0	11.9	.14	.9	6.4	78.6
14	8.5	62.6	25.9	9.7	.32	2.7	8.4	80.9
14	6.9	64.9			.10			
20	6.9	61.3			.11			
23	0	85.1			1.11			
25	11.4	63.4	1.9	15.9	.03	.1	3.3	63.3
26	11.6	69.3	28.2	8.9	.50	3.2	6.4	56.4
27					1.00			
29	14.5	39.0	13.7	.3	.06	45.7	762.0	228.3
40	5.0	80.7	23.9	16.1	.34	1.5	4.4	70.3
46	3.5	63.2	24.5	.6	.12	40.8	340.0	204.2
53	6.7	79.2	17.6	14.1	.24	1.2	5.0	73.3

* Based on oven-dry wt (overnight at 105°C).

† Washington, D. C., 0.1 ppm F, one individual; Los Angeles, Calif., 0.0-0.6 ppm F, in drinking water. All individuals resided in low fluoride area for at least 10 yr prior to removal of calculi. ''Years of residence'' refers to last period of residence.

‡ Colorado Springs, Col., 2.6 ppm F, in drinking water.

§ Same individual. Resided in Washington, D. C. 51 yr prior to fluoridation, and 4 yr thereafter when water contained 1 ppm F.

TABLE II.—*Fat, ash, calcium, phosphorus, and fluoride content of biliary tract calculi* *

Years of residence	Fat	Ash	Ca	P	F
	%				
2	96.5	2.1	1.2	.3	.002
15	97.1	1.2	.6	.2	"
17	52.5	40.3	15.9	.1	.006
28	92.2	5.2	2.2	.6	.003
29	77.6	15.0	4.3	.1	.004
35	89.5	8.6	4.2	.1	.001
39	94.5	3.4			.000
Unknown†	97.0	.2			"
" †	98.7	.1			"

* Based on oven-dry wt (overnight at 105°C).

† Amarillo, Texas, 1.6-3.2 ppm F in the drinking water from 1949 to 1957; remaining samples obtained from Colorado Springs, Col., 2.6 ppm F in the drinking water.

high concentrations of fluoride on forage from air borne sources for four years contained 0.6 p.p.m. fluoride. The small amount of fluoride in biliary tract calculi is in keeping with its low concentration in bile. The extremely low calcium and ash content of biliary calculi (table II) is probably also a factor in their inability to incorporate fluoride.

The data on the fluoride content of urinary and biliary calculi and of bones from similar fluoride areas are summarized in table III on both an oven-dry and ash basis.

As shown in table III, the mean concentration of fluoride was significantly higher in urinary tract calculi than in the bones of individuals from both low fluoride ($p<0.01$) and elevated fluoride areas ($p<0.02$). The mean years of residence of those furnishing the urinary tract calculi and bones were 20.1 and 30.4 years respectively.

The higher concentration of fluoride in the urinary tract calculi may be due, in part, to the higher fluoride concentration reported in urine (*12, 13*) than in blood (*14*). The concentration of fluoride in the urine closely approximates that in the drinking water at equilibrium (*12, 13*). Blood contains about 0.01 p.p.m. fluoride when the drinking water contains 0.1 p.p.m. fluoride and approximately 0.04 p.p.m. fluoride when the water contains 1.0 p.p.m. fluoride (*14*). Although the calculi showed no significant increment in fluoride with an increase in the fluoride content of the drinking water, a four to five fold elevation of fluoride was found in the bones when expressed on either an oven-dry or ash basis. This may be attributed in part to growth and metabolic turnover in bone, whereas, urinary and biliary tract calculi may be considered foreign accretions and hence metabolically inert.

Differences in the composition of calculi and bones may also play an important role in their ability to incorporate fluoride. Thus, the major phase of the inorganic portion of bone is presumed to be a type of hydroxy-apatite (*15, 16*), whereas, eleven different crystalline compounds have been identified in some 600 urinary calculi by Lagergren (*17*) by X-ray diffraction analysis. Although an apatite pattern was seen in seven of nine calculi examined and was the chief component of four in the present study, no relation could be observed between crystal structure and fluoride concentration.

SUMMARY

Thirty-three samples of urinary and nine samples of biliary tract calculi from individuals with known histories of exposure to water-borne fluoride were analyzed for fat, ash, calcium, phosphate and fluoride. (1) Concentration of fluoride in urinary tract calculi was significantly higher than that in the bones. (2) Mean concentration of 0.25 percent fluoride in urinary tract calculi of individuals from low fluoride area was not significantly different from that (0.37 percent) of

TABLE III.—*Fat, ash and fluoride concentration* * *of urinary and biliary calculi, and of bones of individuals from areas containing similar concentrations of fluoride in the drinking water*

	Fat	Ash	Fluoride conc. Oven-dry basis	Fluoride conc. Ash basis
	Low fluoride area (0.0-0.6 ppm F in drinking water)			
Urinary calculi	10.7 ± 7 (13)	60.3 ± 6.1 (13)	.25 ± .05 (16)	.34 ± .07 (13)
Bone	28.3 ± 4.4 (10)	38.0 ± 3.7 (10)	.04 ± .01 (10)	.11 ± .02 (10)
	Elevated fluoride area (2.6 ppm F in drinking water)			
Urinary calculi	7.7 ± 1.1 (15)	68.1 ± 3.1 (15)	.37 ± .08 (17)	.42 ± .09 (15)
Biliary calculi	88.4 ± 5.0 (9)	8.5 ± 4.3 (9)	.002± .0007(9)	.10 ± .03 (9)
Bone	35.6 ± 2.6 (25)	34.8 ± 2.1 (25)	.18 ± .01 (25)	.55 ± .04 (25)

calculi of individuals whose drinking water contained 2.6 p.p.m fluoride. (3) No relation was apparent between the calcium and fluoride concentrations of urinary tract calculi. (4) Fluoride was present in biliary tract calculi in very low concentration (0.000–0.006 percent).

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PATHOLOGIC STUDIES IN MAN AFTER PROLONGED INGESTION OF FLUORIDE IN DRINKING WATER—I. NECROPSY FINDINGS IN A COMMUNITY WITH A WATER LEVEL OF 2.5 P.P.M.

E. F. Geever, N. C. Leone, P. Geiser, and J. Lieberman

The value of artificial fluoridation of drinking water in the prevention of dental caries has been accepted generally. Furthermore, much evidence has been accumulated to indicate that 1 p.p.m. is a safe level that permits beneficial effect and yet avoids possible toxic cumulative effect. This evidence in man is of clinical, statistical and roentgenographic character (*1–8*).

The present study was instituted to furnish information concerning possible pathologic effects in residents of an area with a high natural level of fluoride.

MATERIAL AND METHODS

The series consists of 904 necropsies performed in Colorado Springs between 1947 and 1953 by the senior author (E.F.G.), or by resident physicians in pathology training under his direct supervision. Colorado Springs has a natural fluoride level of approximately 2.5 p.p.m. During the years that the necropsies were performed, the senior author had no special interest in fluorides. Although this might be looked on as desirable in that the necropsies were performed without prejudice in this controversial field, it had the disadvantage that no special attention was given to the bones, a known storehouse for fluorides. Thus, this study was complemented in part II by another undertaken between 1955 and 1957 with detailed examination of bones. The Colorado Springs necropsy protocols were reviewed by the senior author and the diagnoses classified according to: (*a*) the major cause of death; (*b*) contributing causes unrelated to the major cause; (*c*) incidental pathologic condition.

It was hoped that such classification and subsequent analyses might not only indicate whether there was evidence of serious cumulative toxic effects but also whether incidental pathologic processes impairing health were possibly related to the ingestion of fluorides. The material was classified according to residence history. The results were then tabulated and the figures analyzed statistically.

In most instances it was easy to determine the major cause of death from the necropsy protocol, which included a description of the findings on gross and microscopic examination, an abstract of the clinical history and a clinicopathologic summary. In relatively few instances, however, serious coexistent disease required evaluation of all clinical and pathologic features including the character of death. When the decision was made in such instances, the coexistent disease was then listed among the unrelated contributing causes.

Certain other criteria were established arbitrarily and were applied equally to all protocols in each residency group. In a consideration of all deaths caused by new growths with metastases present at necropsy, contributing causes such as the following were considered to be related to the new growth: obstructive conditions from metastases such as pyonephrosis from ureteral involvement due to prostatic cancer and postoperative cardiac failure after surgery for treatment of cancer when the patient had advanced cardiac disease.

Postoperative embolic deaths after cancer surgery were also considered related to the new growth, whether or not metastases were present. If no metastases were found at necropsy and death followed surgery and was due to cardiac failure from advanced cardiac disease, the latter was listed as an unrelated contributing cause and placed in a separate table. Pneumonia was found as a terminal event in many instances where there had been new growths and cardiovascular disease. It was considered a contributing cause related to the major cause unless some special feature was present; that is, lobar distribution of pneumonia and the major disease apparently controlled.

In the group with cardiovascular diseases, whenever a second vascular condition contributed, it might be considered related under certain conditions. Thus, cerebral arteriosclerosis with thrombosis and encephalomalacia accompanying advanced coronary arterial disease would be evaluated as a related cardiovascular disease if the coronary process was considered the major cause of death.

When the necropsy is conducted in a thorough manner, the protocol will almost always contain a number of diagnoses of incidental pathologic conditions in elderly persons. In an attempt to determine the possible effect of fluoride, the necropsy protocols were analyzed first for every condition which might be related. Consideration was given to the possible dangers claimed by opponents of fluoridation (8). Thus, diseases of the thyroid, kidney, liver and pancreas were listed and other specific conditions such as peptic ulcer, gall stones and urinary tract stones. It should be emphasized again that the diseases found in the thyroid, liver, kidney, pancreas, stomach and duodenum were in mild, healing or focal form and often were detected only on microscopic examination. Each patient for whom there was a diagnosis listed in the table of incidental pathologic findings was also affected by one of the major causes of death. For some patients there were, in addition, one or more of the diagnoses listed in the table of contributing causes. Reference has already been made to the fact that in routine necropsies special attention is not given to the bones, and that this study is complemented by another in progress (part II) in which detailed examination of that system was carried out.

Colorado Springs is a place where elderly people from other parts of the country retire, particularly the middle west. In the residence analyses, several groups were considered: a group which had resided in Colorado Springs for less than five years; a second group which had resided in Colorado Springs from 5 to 20 years, and a third group which had resided there for more than 20 years. A fourth group was found also in which the duration of residence in Colorado Springs was unknown.

Table 1.—*Distribution of cases by sex and duration of residence*

Sex	Residence in Colorado Springs				
	Years				
	20+	5-20	<5	Unknown	Sum
Male	174	75	117	46	412
Female	160	55	71	30	316
Total	334	130	188	76	728
	Per cent distribution				
Male	42.2	18.2	28.4	11.2	100.0
Female	50.6	17.4	22.5	9.5	100.0
Total	45.9	17.9	25.8	10.4	100.0

Since the main goal of this study was to determine cumulative toxic effects over long periods, if present, newborn infants and children under 10 years of age were eliminated, and the final analyses consisted of 728 necropsy protocols. There were 188 persons (25.8 percent) who had lived in Colorado Springs for less than 5 years; 130 (17.9 percent) from 5 to 20 years; and 334 (45.9 percent) who had resided in Colorado Springs 20 years and more. In the fourth group in which the duration of residence in Colorado Springs was unknown, there were 76 persons or 10.4 percent (tables 1 and 2).

RESULTS

Pathologic Features. — More detailed analyses were made of the "new growth" and cardiovascular categories after tabulation since they were the most frequent among the major causes of death as is apparent in tables 3 and 4. Of the males in all four groups, bronchogenic and gastrointestinal cancer were most common. In the females, gastrointestinal uterine and breast cancer were most frequent in all four groups.

Before continuing with this detailed analysis, it should be stressed that the statistical interpretation of any differences in disease incidence between residency groups will be discussed under "statistical features."

Cancer of the kidney and bones was as uncommon in the group with residency in Colorado Springs for over 20 years as in the other three categories: there were 3 instances of renal, and 3 of bone cancer among the 334 persons in the over 20 years' residency group. Among the

Table 2.—*Distribution of cases by age, sex, and duration of residence*

Age at death: years	Residence in Colorado Springs					Per cent distribution				
	Years									
	20+	5-20	<5	Unknown	Sum	20+	5-20	<5	Unknown	Sum
10-19	—	3	4	1	8	—	2.3	2.1	1.3	1.1
20-29	10	1	8	6	25	3.0	0.8	4.3	7.9	3.4
30-39	7	3	11	8	29	2.1	2.3	5.9	10.5	4.0
40-49	27	15	27	14	83	8.1	11.5	14.4	18.4	11.4
50-59	55	20	43	13	131	16.5	15.4	22.9	17.1	18.0
60-69	70	34	49	15	168	21.0	26.2	26.1	19.7	23.1
70-79	116	39	35	16	206	34.7	30.0	18.6	21.1	28.3
80+	49	15	11	3	78	14.7	11.5	5.9	3.9	10.7
Sum	334	130	188	76	728	100.0	100.0	100.0	100.0	100.0
					Male					
10-19	—	—	3	1	4	—	—	2.6	2.2	1.0
20-29	5	—	4	3	12	2.9	—	3.4	6.5	2.9
30-39	3	2	6	5	16	1.7	2.7	5.1	10.9	3.9
40-49	15	10	15	10	50	8.6	13.3	12.8	21.7	12.1
50-59	28	11	31	6	76	16.1	14.7	26.5	13.0	18.4
60-69	42	23	32	11	108	24.1	30.7	27.4	23.9	26.2
70-79	55	22	22	9	108	31.6	29.3	18.8	19.6	26.2
80+	26	7	4	1	38	14.9	9.3	3.4	2.2	9.2
Sum	174	75	117	46	412	100.0	100.0	100.0	100.0	100.0
					Female					
10-19	—	3	1	—	4	—	5.5	1.4	—	1.3
20-29	5	1	4	3	13	3.1	1.8	5.6	10.0	4.1
30-39	4	1	5	3	13	2.5	1.8	7.0	10.0	4.1
40-49	12	5	12	4	33	7.5	9.1	16.9	13.3	10.4
50-59	27	9	12	7	55	16.9	16.4	16.9	23.3	17.4
60-69	28	11	17	4	60	17.5	20.0	23.9	13.3	19.0
70-79	61	17	13	7	98	38.1	30.9	18.3	23.3	31.0
80+	23	8	7	2	40	14.4	14.5	9.9	6.7	12.7
Sum	160	55	71	30	316	100.0	100.0	100.0	100.0	100.0

Table 3.—*Major cause of death by length of residence in Colorado Springs*

Cause	Total				Male				Female			
	20+	5-20	<5	Unknown	20+	5-20	<5	Unknown	20+	5-20	<5	Unknown
New growth	111	26	50	23	52	12	28	10	59	14	22	13
Accident	14	7	7	7	7	3	7	7	7	4	0	—
Leukemia	3	2	3	1	2	1	2	—	1	1	1	1
Anemia	3	—	2	—	3	—	1	—	—	—	1	—
T. B.	11	5	16	4	5	4	11	4	6	1	5	—
Other	16	8	17	2	4	2	7	—	12	6	10	2
Other diseases by systems:												
Cardiovascular	109	55	60	27	60	37	40	19	49	18	20	8
Respiratory	20	8	11	4	12	5	9	1	8	3	2	3
Digestive tract	26	8	8	3	16	6	5	2	10	2	3	1
Liver	4	6	—	0	3	4	—	—	1	2	—	0
Nervous system	2	1	7	3	—	—	2	2	2	1	5	1
Urogenital tract	15	4	7	2	10	1	5	1	5	3	2	1
Total	334	130	188	76	174	75	117	46	160	55	71	30

Table 4.—*Major cause of death by length of residence in Colorado Springs (percent distribution)*

Cause	Total				Male				Female			
	20+	5-20	<5	Unk.	20+	5-20	<5	Unk.	20+	5-20	<5	Unk.
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
New growth	33.2	20.0	26.6	30.3	29.9	16.0	23.9	21.7	36.9	25.5	31.0	43.4
Accident	4.2	5.4	3.7	9.2	4.0	4.0	6.0	15.2	4.4	7.3	—	—
Leukemia	0.9	1.5	1.6	1.3	1.1	1.3	1.7	—	0.6	1.8	1.4	3.3
Anemia	0.9	—	1.1	—	1.7	—	0.9	—	—	—	1.4	—
T. B.	3.3	3.8	8.5	5.3	2.9	5.3	9.4	8.7	3.8	1.8	7.0	—
Other	4.8	6.2	9.0	2.6	2.3	2.7	6.0	—	7.5	10.9	14.1	6.7
Other diseases by symptoms:												
Cardiovascular	32.6	42.3	31.9	35.5	34.5	49.3	34.2	41.3	30.6	32.7	28.2	26.8
Respiratory	6.0	6.2	5.9	5.3	6.9	6.7	7.7	2.2	5.0	5.5	2.8	10.0
Digestive tract	7.8	6.2	4.3	3.9	9.2	8.0	4.3	4.3	6.3	3.6	4.2	3.3
Liver	1.2	4.6	—	—	1.7	5.3	—	—	0.6	3.6	—	—
Nervous system	0.6	0.8	3.7	3.9	—	—	1.7	4.3	1.3	1.8	7.0	3.3
Urogenital tract	4.5	3.1	3.7	2.6	5.7	1.3	4.3	2.2	3.1	5.5	2.0	3.3

188 persons in the less than 5 years' duration residency group, there were 2 instances of renal and 2 of bone cancer. There were no instances of primary hepatic cancer in this group, and two in the over 20 years' residency series. Occasionally, a benign new growth resulted in death. For example, the postoperative cardiac failure or embolism was sometimes responsible for death after hysterectomy for fibromyomata or lobectomy for bronchial adenoma.

In the cardiovascular system of all groups, the most common condition causing death was coronary arterial disease. The clinicopathologic features in 100 consecutive fatal instances in this series were reported elsewhere by the senior author (*10*). Cerebral vascular, hypertensive cardiorenal and aortic disease with rupture were fairly frequent causes of death in all four groups. The coronary arterial disease deaths were 42, 28, 31 and 9 respectively in the over 20 years, 5 to 20, less than 5 years, and unknown, residency groups. Hypertensive cardiorenal disease occurred in 16, 9, 5 and 4 instances respectively. The cerebral vascular diseases were 23, 2, 6 and 1 respectively, and in the over 20 years' duration residency group of 23, 17 of them occurred in women.

In the major causes of death from conditions in the urogenital tract, apart from neoplasms, the men in the over 20 years' duration residency group were affected in 10 instances, 8 from prostatic hypertrophy. The enlarged prostate usually caused a train of obstructive and inflammatory changes in the urinary bladder, ureters and kidneys. Pyelonephritis and pyonephrosis as a result of the prostatic condition often resulted in death due to uremia. There were two independent renal conditions as major causes of death in this over 20 years' duration residency group of 10: one was chronic glomerulonephritis with death at 51 years of age, and one with chronic pyelonephritis and death at 74 years of age. There were five deaths from urogenital causes in the men in the under 5 years' residency group: three were due to prostatic disease and two to primary renal disease; that is, one with glomerulonephritis and death at 52 years of age, the other with congenital polycystic kidneys and death at the age of 45.

Among the women, urogenital tract disease caused five deaths in the over 20 years' duration residency group; one from arteriolosclerotic nephrosclerosis plus pyelonephritis at 72 years of age, and four with pyelonephritis at ages 62, 71, 73 and 82 years. This is in contrast with two deaths in the under 5 years' exposed group, one with chronic glomerulonephritis with death at 18 years of age and the other with pyelonephritis and death occurring at 65 years.

Table 5 indicates the incidental pathologic conditions found. Those placed under the heading of thyroid included nodular goiter, fetal or other type adenomas, and miscellaneous pathologic changes such as fibrosis, cystic or calcific changes.

The incidental renal pathologic conditions included vascular changes; that is, arterial changes or arteriosclerosis or both, tubular degeneration, inflammatory processes, adenomas and cysts. Among the hepatic incidental diagnosis were cirrhosis, noncirrhotic inflammatory changes, adenomas or hemangiomas, cysts and fatty metamorphosis. The other headings are self-explanatory.

Statistical Features.—Many statisticians have demonstrated that necropsy material can supply only limited information and has certain inherent biases. However, the reporting on the source material being considered here is uniform because the necropsies were performed by one pathologist or by resident physicians in training under his direction. He was responsible for reviewing all necropsies which he did not perform himself. Secondly, all 728 necropsies that were done in the period under consideration were studied in this report.

This report deals essentially with a trichotomy of the total series and is based on an attribute (residence history) which is independent of the inherent weaknesses of necropsy series. There is no reason to believe that either group within the series was favored or penalized since the pathologist was unaware of the residence history when the necropsies were performed. He was also unaware of the ultimate use of the protocols in this report. It is, therefore, believed that the comparisons between groups within the series are valid.

Age, sex and residence characteristics are given in tables 1 and 2. Of the 334 instances in the over 20 years' duration residency group, further analysis revealed that 234 had been residents of Colorado Springs for more than 30 years and 149 had been residents for more than 40 years. From table 2 it is apparent that 49.4 percent of the persons in the over 20 years' residency group were 70 years of age and older, 41.5 percent in the 5 to 20 year's residency group, 24.5 percent in the under 5 years', and 25.0 percent in the unknown residency groups. Thus

TABLE 5.—*Incidental pathologic findings at necropsy*

	20+		5-20 Years		<5 Years		Unknown	
	Male	Female	Male	Female	Male	Female	Male	Female
Thyroid disease	19	60	12	16	12	24	12	13
Gall stones	20	42	11	16	7	11	2	5
Renal disease	77	72	32	21	46	29	15	15
Urinary tract stones	7	5	1	2	4	1	2	1
Hepatic disease	31	27	11	9	29	6	7	8
Peptic ulcer	7	8	5	0	4	3	3	2
Diabetes mellitus*	1	3	1	0	0	1	0	1
Pancreatitis	7	7	1	2	3	0	1	1
Total	169	224	74	66	105	75	42	46

*Clinical diagnosis.

comparison between groups must be made with this fact in mind.

Tables 3 and 4 list the data with respect to the major cause of death. One out of every four necropsy protocols showed the major cause of death to be neoplasm. There were 210 such protocols in 728, or 28.8 per cent. Diseases of the cardiovascular system comprised 34.5 percent. New growths were more frequent in females than in males; that is, 34.2 percent and 24.8 percent. The difference of 9.4 percentage points is statistically significant. The explanation is probably related to the difference in the age distribution among males and females. There were 35.4 percent of the men who were 70 years and over, whereas 43.7 per cent of the women were 70 years of age and over. In table 4 it can be seen that in the 5 to 20 years' duration residency group, the total of the deaths from new growths and cardiovascular conditions was 62.3 per cent, approximately the same as the long term total for the two groups of diseases; that is, 65.8 percent for the over 20 years' duration residency group. However, the total for the new growths was 20.0 percent or 13.2 percentage points below the 33.2 percent of the over 20 years' duration residency group. The difference is statistically significant.

Similarly, the difference of 9.7 percentage points between the 42.3 percent deaths of the 5 to 20 years' residency group from diseases of the cardiovascular system and the 32.6 percent figure in the long term group; that is, the over 20 years' duration residency group is statistically significant.

The explanation for these differences probably lies in the difference in the age distributions and the fact that there were fewer persons in the medium duration residency group; that is, 26 out of 130 as compared with 111 out of 334. Obviously, it would be as unreasonable to attribute an apparent beneficial effect to fluoride in reducing deaths from cardiovascular conditions in the long term residency group as it would to attribute to it a deleterious effect in elevating deaths from new growths.

The same reasoning is applicable, based on differences in age distribution and small numbers, in explanation of the differences noted on detailed analysis of disease in the previous section on pathologic features. It is also apparent that in the medium duration residency group (5 to 20 years), the percentage of deaths from new growths was less than was the percentage in the under 5 years' duration group. Similarly, the medium duration group had a higher percentage of deaths from cardiovascular diseases than did the under 5 years' residency group.

There was relatively little difference in the percentages of new growth and cardiovascular deaths between the long term (over 20 years) and short term (less than 5 years) groups.

The less than 5 years' residency group revealed a high percentage of deaths from tuberculosis, but this was not unexpected because Colorado Springs at the time the necropsies were performed was still a tuberculosis treatment center.

Table 6 lists the contributing causes of death. Six patients in the over 20 years' residency group had two unrelated contributing causes, 40 patients had one, and 288 had none. Six patients were found in the under 5 years' residency group with two contributing causes, 19 had one contributing cause, and the remaining 163 had none. In the other two residency categories, no patient had more than one contributing cause. Cardiovascular diseases predominated or were as frequent as any other disease in any of the four residency groups. There was no greater incidence of cardiovascular disease in the 5 to 20 years' group than in the over 20 years' duration residency group to confirm that shown in the major causes of death in table 4. The pancreatitis noted in the over 20 years' duration residency group will be referred to later.

In the over 20 years' duration residency group, no incidental pathologic conditions were found in 93 patients (27.8 percent) in the categories listed in table 5; 129 (38.6 percent) had one and 112 (33.5 percent) had two or more. In the 5 to 20 years' duration residency group, 39 patients (30.0 percent) had none, 49 (37.6 percent) had one, and in 42 (32.3 percent) two or more incidental pathologic conditions were noted. In the less than 5 years' residency group, 66 persons (35.1 percent) had none, 75 (39.9 percent) had one, and 47 (25.0 percent) had two or more conditions diagnosed. In the fourth group, residency unknown, 29 patients (38.2 percent) had none, 21 (27.6 percent) had one, and in 26 (34.2 percent) two or more incidental pathologic changes were noted.

The differences between residency

TABLE 6.—*Total contributing causes unrelated to the major cause*

	20+		5-20 Years		<5 Years		Unknown	
	Male	Female	Male	Female	Male	Female	Male	Female
New growth	1	0	0	0	2	0	1	2
Accident	0	0	0	0	0	0	0	0
Leukemia	0	0	0	0	0	0	0	0
Anemia	0	0	0	0	0	0	0	0
T. B.	1	1	0	0	1	1	0	0
Others:								
Thyroid	0	2	0	0	0	0	0	1
Pancreatitis	4	0	0	0	0	0	0	0
Diabetes m.*	2	1	0	0	0	3	1	0
Miscellaneous	1	0	1	0	1	4	0	0
Other diseases by systems:								
Cardiovascular	14	9	5	3	6	4	1	2
Respiratory	3	1	2	0	2	2	0	0
Digestive	2	2	1	1	2	0	0	0
Liver	0	2	0	0	2	0	0	1
Nervous system	1	1	0	0	0	0	0	0
Urogenital	1	3	0	0	1	0	1	2
Total	30	22	9	4	17	14	4	8

*Clinical diagnosis.

groups as a whole were not significant statistically. However, a statistically significant difference was observed in the percentage in whom gall stones was noted between the over 20 years' duration residency group and the group residing in Colorado Springs less than 5 years. Gall stones occurred in 62 instances, 20 men and 42 women, for a total of 18.6 percent in the former, and 18 instances, 7 men and 11 women or 9.6 percent in the latter or short-term residency group. This difference can be explained on the basis of the difference in median ages in the two groups: in the long term; that is, over 20 years' duration residency group, the median age of the men was 68 years, the women 70 years. In the less than 5 years' duration residency group, the median age was 59 years for the men and 61 for the women.

Mentzer, (*11*), in 633 necropsies in Rochester, Minn., found gall stones in 22 percent of the adults over 21 years of age as against the 18.6 percent quoted for the long-term residents in the present study. Analysis of our series by decades and sex reveals that 52.5 percent of the women in the over 20 years' duration residency group died at the age of 70 or older. Gall stones were found at necropsy in 22 of these women. There were 14 instances of gall stones among 61 women between 70 and 79 years of age, and eight among 23 women 80 years of age and over. The corresponding percentages were 23.0 and 34.8 percent. This could be compared with figures of 26.8 percent and 50 percent from Ludlow's series in Cleveland (*12*). He found the two values mentioned in women in the decades 71 to 80 and 81 to 90 years, respectively. Both cities, Rochester, Minn. and Cleveland, had little or no fluoride in their drinking water at the time of the studies reported by Mentzer and Ludlow.

Chemical analyses of gall stones were reported from communities, including Colorado Springs, Colo., with elevated fluoride levels in the drinking water. Zipkin, Lee, and Leone (*13*) found no significant increase in fluoride in these calculi.

It is generally accepted that pancreatitis is often associated with gall bladder disease and calculi. In analyzing the instances of pancreatitis in the over 20 years' duration residency series, either as a major or contributing cause of death, or as an incidental finding, 12 out of 21 instances of pancreatitis were accompanied by gall stones or cholecystitis or both. Thus, the apparent increase in pancreatitis as seen in tables 5 and 6 can be related directly to the gall stones and to the older median age of the long term (over 20 years' duration residency) group.

In conclusion, the overall comparison of incidental findings in the various residency groups revealed a surprising degree of uniformity. Usually, in a table with so many comparisons possible, many more striking differences might be expected.

SUMMARY

1. Necropsies were performed or supervised by one of us (E.f.g.) on 904 patients between 1947 and 1953 in Colorado Springs, Colo. This is a community in which there was a natural fluoride level of 2.5 p.p.m. in the drinking water during that period.

2. The results of reexamination of the protocols and classification of the data into the major cause of death, contributing causes and incidental pathologic conditions were tabulated.

3. The residence histories after eliminating deaths among the newborn and among children under 10 years of age, revealed four groups: 334 persons who had resided in Colorado Springs for more than 20 years; 130 who had resided there from 5 to 20 years; 188 under 5 years, and 76 in whom the residence history was incomplete. This study of the pathologic findings was thus based on 728 necropsies.

4. Comparative statistical analyses of the pathologic findings revealed no significant differences which could be related to prolonged residence in this environment.

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FLUORIDE DEPOSITION IN HUMAN BONES AFTER PROLONGED INGESTION OF FLUORIDE IN DRINKING WATER

I. Zipkin, F. J. McClure, N. C. Leone, and W. A. Lee

In conjunction with microscopic examination of human bones for possible effects of prolonged ingestion of fluoride in drinking water by Geever and associates (pp. 452-457 in this volume) essentially the same bones were analyzed for ash and fluoride. These bones were obtained at autopsy from 69 persons, 36 of whom were exposed to 1.0 to 4.0 p.p.m. fluoride for 10 to 76 years. The remaining 33 had lived in areas with less than 1.0 p.p.m. fluoride for 10 to 87 years.

This study represents the first systematic survey of the fluoride concentration of bones of individuals exposed to various concentrations of fluoride in drinking water. Previous studies of exposure to high levels of fluoride, recently reviewed by McClure and associates (pp. 741-746 of this issue), have dealt with no more than four cases (*1–5*). More extensive data have been reported on the fluoride concentration of selected bones of individuals in low-fluoride areas (*6–8*).

Most of the persons whose bones were examined in the present studies died suddenly, the chief causes being trauma, coronary heart disease, and cerebrovascular accidents. Although bones of persons with chronic illness or diseases known to affect bone structure were excluded from the microscopic study, they were included in the fluoride deposition study so that the effect of such conditions might be observed. The fluoride analysis thus included bones from 3 individuals with a malignancy and 3 with renal disease.

The 69 individuals in the fluoride deposition study, 40 men and 29 women aged 26 through 90 years, provided 190 bones and 64 specimens of intervertebral cartilage. Approximately 80 percent of these bones and cartilage specimens were also examined histologically. The bones, consisting of a portion of the iliac crest, the lumbar vertebra, and the sixth rib, were fixed in 10 percent formalin at autopsy.

The intervertebral cartilage was separated from the vertebra, and all specimens were cleaned of adhering soft tissues. Each sample was dried overnight at 105° C., broken into pieces, and extracted with alcohol for 8 hours and with ether for 4 hours. After the sample was ground to pass a 60-mesh sieve, a portion was ashed for 3 hours at 550° C. and analyzed for fluoride (*9–11*). The significance of the differences in means was calculated according to Fisher's *t* test for paired values. The sodium, potassium, calcium, magnesium, phosphorus, carbon dioxide, and citrate contents of these tissues will be reported later.

BONE FLUORIDE

The percentages of ash and fluoride in dry, fat-free bones for each individual in the study, grouped according to the level of fluoride in the drinking water, are recorded in tables 1 through 4. Ages and years of residence are also given, but it is not possible to detect any definite relation between the fluoride concentration and these factors.

The fluoride values for the 15 bones from the individuals who had a malignancy or renal disease were within the variations encountered for others in the same group (subject No. 5 in table 1; subjects Nos. 8, 10, and 11 in table 3; iliac crest, subject No. 1 in table 4) or slightly higher (subject No. 2 in table 2; rib and vertebra, subject No. 1 in table 4). No significance can be attached to the somewhat higher values since they occurred in individuals from the two smaller groups (tables 2 and 4). Moreover, approximately a tenfold increase in fluoride concentration of the bones from the lowest to the highest fluoride areas (tables 1 and 4) was seen without any apparent tissue damage.

LESS THAN 1 P.P.M. FLUORIDE

Individuals exposed to less than 1 p.p.m. fluoride were residents of several localities, with fluoride concentrations in the drinking water varying from 0.1 p.p.m. in New York City to 0.4 p.p.m. in San Antonio, Tex. The mean was 0.2 p.p.m. Five persons in this group, aged 32 through 74 years, drank water fluoridated to 1 p.p.m. fluoride for 2 to 4 years. The fluoride content of the bones of these five was within the variations encountered in other samples in this group (table 1).

On a dry, fat-free basis, the mean concentrations of fluoride in the various bones of individuals exposed to less than 1 p.p.m. fluoride were similar, 0.041, 0.050, and 0.054 percent for the iliac crest, the rib, and the vertebra, respectively. As expected, the fluoride concentrations of the rib and the vertebra were generally highest in the individuals from San Antonio.

Five samples of sternum from this group varied from 0.040 to 0.101 percent fluoride with a mean, ± standard error, of 0.066 ± 0.012 percent (not including one inordinately high value of 0.229 percent fluoride). On an ash basis, they ranged from 0.078 to 0.188 with a mean of 0.134 percent. The mean concentration of fluoride in the ash of the vertebra (0.112 ± 0.010 percent) was similar to that of the sternum ash (0.134 ± 0.023 percent), but it was significantly higher than that in the ash of the iliac crest (0.072 ± 0.006 percent, $P<0.01$) or in the rib (0.086 ± 0.008 percent, $P<0.05$).

While ash concentrations of the vertebra and the sternum were similar (49.0 percent and 49.4 percent), they were significantly lower than that of the iliac crest (57.4 percent, $P<0.01$) or the rib (58.8 percent, $P<0.01$).

FLUORIDE LEVEL OF 1 P.P.M.

A second group, composed of 5 persons, were residents of Grand Rapids, Mich., where the water supply was fluoridated to 1 p.p.m. in January 1945. The autopsy specimens were obtained between November 1956 and January 1957; thus exposure to 1 p.p.m. fluoride was for a maximum of 12 years during the last years of life.

As with the low-fluoride group, no differences were found in the concentration of flouride in the various bones of this group (table 2). On a dry, fat-free basis, the mean percentages were 0.138 for the iliac crest, 0.139 for the rib, and 0.160 for the vertebra. The fluoride concentrations of the ash of the iliac crest (0.225 ± 0.026 percent) and of the rib (0.241 ± 0.030 percent) were similar, but they were significantly lower than that of the vertebra (0.323 ± 0.043 percent, $P<0.05$). Appreciable amounts of fluoride thus accrued during the last 10 to 12 years of life of these 5 individuals aged 64 through 85 years.

For the 5 exposed to 1 p.p.m. fluoride, the concentrations of ash in the iliac crest (61.2 percent) and the rib (57.4 percent) were similar but significantly higher than that of the vertebra (50.0 percent, $P<0.01$).

Table 1.—*Percentage ash and fluoride in dry, fat-free bones of individuals ingesting drinking water containing less than 1 p.p.m. fluoride*

Subject No.	Sex	Age (years)	Residence (years)	Iliac crest		Rib		Vertebra	
				Ash	Fluoride	Ash	Fluoride	Ash	Fluoride
		0.1 ppm fluoride							
1	Male	27	27	56. 8	0. 019	61. 4	0. 020	50. 2	0. 031
2	Male	32	12	56. 2	. 044	56. 9	. 037	50. 4	. 054
3	Male	35	35	58. 1	. 045	59. 4	. 043	43. 7	. 043
4	Female	38	12	--------	--------	--------	--------	52. 2	. 024
5	Male	47	10	--------	--------	--------	--------	49. 9	. 056
6	Female	47	15	57. 6	. 020	57. 9	. 024	46. 6	. 019
7	Male	50	25	59. 6	. 030	60. 9	. 024	51. 7	. 026
8	Female	55	20	58. 9	. 038	53. 9	. 043	43. 8	. 036
9	Male	56	20	52. 6	. 046	57. 8	. 047	47. 9	. 057
10	Male	62	62	54. 9	. 032	57. 6	. 028	50. 9	. 038
11	Male	62	62	58. 8	. 024	57. 3	. 031	46. 8	. 028
12	Male	65	41	54. 2	. 034	54. 0	. 062	43. 2	. 034
13	Male	66	15	57. 6	. 028	61. 9	. 039	45. 7	. 026
14	Female	68	10	62. 9	. 073	65. 5	. 077	55. 7	. 079
15	Male	69	10	58. 3	. 035	60. 0	. 037	50. 5	. 034
16	Male	70	60	54. 8	. 032	60. 2	. 039	44. 3	. 042
17	Female	73	32	54. 0	. 048	57. 6	. 060	44. 6	. 055
18	Female	82	40	56. 8	. 044	58. 8	. 037	--------	--------
19	Male	87	87	59. 2	. 055	59. 8	. 062	46. 9	. 059
		0.2 ppm fluoride							
20	Female	32	10	--------	--------	68. 4	0. 023	53. 6	0. 038
21	Male	32	[1] 32	58. 8	0. 053	64. 1	. 031	51. 7	. 043
		0.3 ppm fluoride [2]							
22	Female	48	48	--------	--------	60. 5	0. 048	--------	--------
23	Male	53	24	59. 7	0. 046	54. 4	. 056	47. 3	0. 050
24	Male	59	57	--------	--------	--------	--------	46. 2	. 093
25	Female	74	25	59. 0	. 078	57. 9	. 089	43. 7	. 077
		0.1–0.4 ppm fluoride							
26	Male	32	32	--------	--------	56. 2	0. 062	45. 4	0. 061
27	Female	40	10	--------	--------	60. 6	. 071	49. 8	. 111
28	Male	44	10	--------	--------	53. 4	. 039	46. 4	. 042
29	Male	57	34	--------	--------	60. 2	. 039	44. 9	. 040
30	Male	59	10	--------	--------	54. 4	. 078	48. 5	. 094
31	Male	64	10	--------	--------	60. 4	. 064	51. 4	. 075
32	Male	71	10	--------	--------	60. 0	. 069	45. 3	. 083
33	Male	74	20	--------	--------	51. 3	. 106	49. 8	. 119
	Mean	55. 5	28. 1	57. 4	. 041	58. 8	. 050	49. 0	. 054
	SE	2. 8	3. 4	. 3	. 003	. 7	. 004	. 6	. 005

[1] Resident of Baltimore, Md.; water fluoridated to 1 ppm in November 1952; received fluoridated water for 2 years.

[2] Residents of Washington, D. C.; water fluoridated to 1 ppm in June 1952; received fluoridated water 4, 2, 3, and 3 years, respectively.

FLUORIDE LEVEL OF 2.6 P.P.M.

Colorado Springs, Colo., and Amarillo, Tex., were the residences of 27 subjects. Colorado Springs has had a uniform and verified history of 2.5 p.p.m. fluoride within narrow limits for some 75 years (*12, 13*). The fluoride level of the water of Amarillo from 1934 to 1948 ranged from 3.6 p.p.m. to 6.2 p.p.m. (*14*). According to a personal communication from the city chemist, 33 new wells were added between 1948 and 1953, and the fluoride level ranged from 2.6 to 3.2 p.p.m. In 1955 and 1956 the water supply of Carson County, which probably contains less than 1 p.p.m. fluoride inasmuch as Dean (*15*) considered the incidence of mottled enamel virtually negative, was developed to augment that of Amarillo. In 1957, 92 wells were

Table 2.—*Percentage ash and fluoride in dry, fat-free bones of individuals ingesting drinking water containing 1 p.p.m. fluoride, Grand Rapids, Mich.*[1]

Subject No.	Sex	Age (years)	Residence (years)	Iliac crest		Rib		Vertebra	
				Ash	Fluoride	Ash	Fluoride	Ash	Fluoride
1	Female	64	12	----------	----------	57.8	0.135	45.9	0.130
2	Female	64	15	61.9	0.176	59.7	.195	48.4	.238
3	Male	82	10	59.5	.137	54.5	.119	51.3	.140
4	Female	83	20	61.1	.137	----------	----------	53.3	.135
5	Male	85	50	62.2	.100	57.4	.106	50.9	.159
	Mean	75.6	21.5	61.2	.138	57.4	.139	50.0	.160
	SE	1.5	7.4	.6	.016	1.1	.020	1.3	.020

[1] Fluoride added to drinking water January 1945. Subjects received fluoridated water for 12 years with exception of male aged 82 years.

Table 3.—*Percentage ash and fluoride in dry, fat-free bones of individuals ingesting drinking water containing a mean of 2.6 p.p.m. fluoride, Colorado Springs, Colo., and Amarillo, Tex.*

Subject No.	Sex	Age (years)	Residence (years)	Iliac crest		Rib		Vertebra	
				Ash	Fluoride	Ash	Fluoride	Ash	Fluoride
1	Male	36	10	59.6	0.095	57.8	0.106	47.3	0.098
2	Male	36	36	58.9	.092	61.1	.103	49.7	.145
3	Female	47	10	57.3	.219	59.7	.194	54.3	.239
4	Female	47	15	61.4	.481	66.7	.370	56.8	.521
5	Male	50	10	57.9	.129	58.3	.199	52.7	.179
6	Female	53	22	61.2	.111	62.2	.134	52.4	.163
7	Female	54	20	62.0	.247	65.0	.179	53.8	.332
8	Male	54	20	----------	----------	56.1	.269	43.7	.325
9	Male	56	10	57.3	.141	55.1	.104	44.9	.150
10	Male	57	46	58.2	.288	59.5	.278	32.7	.197
11	Male	58	25	----------	----------	58.9	.391	45.0	.334
12	Female	63	25	60.0	.297	58.0	.279	47.8	.305
13	Male	65	30	60.0	.268	56.9	.276	47.9	.299
14	Female	66	10	62.7	.214	59.4	.194	44.3	.244
15	Female	69	28	59.9	.458	58.2	.453	50.7	.548
16	Male	71	36	53.3	.454	57.2	.363	47.2	.400
17	Female	75	16	60.8	.237	59.3	.244	42.6	.262
18	Male	76	76	61.6	.371	55.5	.347	54.0	.346
19	Male	79	40	61.7	.176	59.4	.161	47.3	.189
20	Female	80	13	60.4	.190	59.5	.205	50.2	.293
21	Female	81	52	60.1	.321	59.3	.293	36.6	.264
22	Female	82	76	56.4	.334	52.3	.338	46.0	.382
23	Female	83	54	62.0	.325	62.4	.267	51.9	.367
24	Female	83	60	58.6	.348	62.4	.373	56.1	.363
25	Male	84	32	60.9	.154	58.6	.121	53.7	.177
26	Female	84	62	59.3	.431	61.7	.443	45.4	.321
27	Male	90	55	60.9	.304	62.2	.245	48.1	.295
	Mean	65.9	32.9	59.7	.267	59.7	.257	48.3	.286
	SE	3.0	4.0	.4	.023	.6	.020	1.1	.023

TABLE 4.—*Percentage ash and fluoride in dry, fat-free bones of individuals ingesting drinking water containing a mean of 4.0 p.p.m. fluoride, Lubbock, Tex.*

Subject No.	Sex	Age (years)	Residence (years)	Iliac crest		Rib		Vertebra	
				Ash	Fluoride	Ash	Fluoride	Ash	Fluoride
1	Male	26	17	58. 6	0. 445	62. 8	0. 458	51. 2	0. 564
2	Male	53	10	60. 8	. 374	54. 5	. 401	47. 5	. 348
3	Male	71	48	62. 1	. 542	53. 3	. 449	59. 9	. 470
4	Female	74	10	58. 1	. 291	59. 4	. 284	44. 3	. 261
	Mean	56	21. 3	59. 9	. 413	57. 5	. 398	50. 7	. 411
	SE	11	9. 1	. 9	. 053	2. 2	. 040	3. 4	. 068

pumping into the Amarillo reservoir, and the fluoride content averaged 1.6 p.p.m. From 1948 to 1957, the fluoride level of the drinking water in Amarillo averaged 2.8 p.p.m. and the weighted mean for the two cities was 2.6 p.p.m.

For the 27 individuals, the mean fluoride concentration, on a dry, fat-free basis, of the iliac crest was 0.267 percent; of the rib, 0.257 percent; and of the vertebra, 0.286 percent (table 3). On an ash basis, the mean concentration of fluoride in the vertebra (0.598±0.015 percent) was significantly higher than that in the iliac crest (0.450±0.040 percent, $P<0.01$) or in the rib (0.432±0.036 percent, $P<0.01$). The concentration of ash in the iliac crest and the rib (59.7 percent) was significantly higher than that in the vertebra (48.3 percent, $P<0.01$).

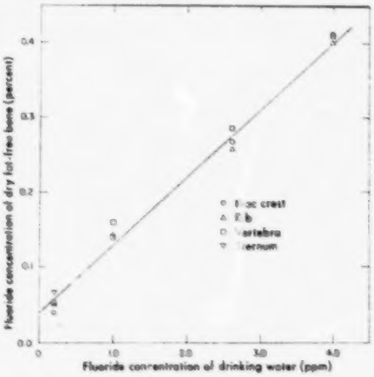
FLUORIDE LEVEL OF 4.0 P.P.M.

Prolonged exposure to about 4.0 p.p.m. fluoride in the drinking water took place in Lubbock, Tex., now the largest community in the United States reported to have this much fluoride. Because of the unusually high fluoride content of its drinking water, Lubbock, as well as Amarillo, has been the scene of a number of previous studies of the effects of fluoride (*14–18*). The water fluoride of Lubbock up to 1948 ranged from 3.5 to 4.4 p.p.m. (*14*). In a recent letter from the Lubbock City-County Health Department, the fluoride content of the water supply from storage reservoirs was reported to have varied from 3.1 to 5.2 p.p.m. from 1948 through 1955, with a mean of 4.0 p.p.m. The four subjects from Lubbock were autopsied from May through July 1955.

For the Lubbock residents as for the other subjects, the fluoride concentrations of the dry, fat-free iliac crest, rib, and vertebra were not significantly different, being 0.413, 0.398, and 0.411 percent, respectively. Although the concentration of fluoride in the ash of the vertebra (0.802±0.109 percent) was greater than that of the iliac crest (0.687±0.081 percent) or that of the rib (0.629±0.095 percent), the difference was not significant ($P>0.05$). The ash content of the iliac crest (59.9 percent) and that of the rib (57.5 percent) were similar but higher than the ash content of the vertebra (50.7 percent). An unusually high value of 59.9 percent ash for a specimen of vertebra prevented any level of significance being attached to the difference in the mean ash content of the various bones.

GROUP COMPARISON

To summarize these data, the mean concentrations of fluoride in the various bones were plotted against the fluoride level of the drinking water to which the individuals were exposed (see chart). The result indicates that the relation between fluoride in the bones and fluoride in the drinking water up to 4.0 p.p.m. is adequately described by a straight line function.



Relation of fluoride in dry, fat-free bones to fluoride in drinking water.

OTHER FINDINGS

The concentration of fluoride in the dry, fat-free intervertebral cartilage also appeared to increase somewhat with elevated levels of waterborne fluoride (table 5). Calcification of this tissue, however, appears not to have been affected by the fluoride concentration of the drinking water.

In addition to the fluoride and ash analyses reported in the preceding tables, it may be of interest to note that the fat content of neither the bones nor the intervertebral cartilage was related to the fluoride content of the drinking water. The mean concentrations of fat in the oven-dry bones had the following range:

	Lowest	*Highest*
Iliac crest	23. 2±5. 7	35. 3±2. 6
Rib	23. 4±3. 6	30. 2±5. 7
Vertebra	32. 2±7. 6	43. 8±4. 3
Sternum [1]	41. 3±4. 3	41. 3±4. 3
Intervertebral cartilage	1. 8± . 2	3. 3± . 8

[1] Only one fluoride group examined.

DISCUSSION

The fluoride concentrations in the bones examined in the present study from both low-fluoride areas and high-fluoride areas approximate those observed in the previous limited studies (tables 6 and 7). For one resident of London, a low-fluoride area, a very high value of 0.687 percent fluoride in rib ash was obtained (*7*). The highest concentrations of fluoride found in the present study in bones of individuals who had been drinking water containing less than 1.0 p.p.m. fluoride was 0.428 percent in a sample of ashed sternum, and the next high value was 0.238 percent fluoride for a sample of vertebral ash. In considering the data for high-fluoride areas, some reservations must be attached to the high values presented by Kilborn and associates (*6*), since these results may have been due to the subnormal living standards and the

Table 5.—*Percentage ash and fluoride in intervertebral cartilage in relation to fluoride level of drinking water*

Fluoride level of water (ppm)	Number of samples	Dry, fat-free basis		Ash basis
		Ash	Fluoride	Fluoride
<1.0	29	4. 8±0. 3	0. 003±0. 000	0. 049±0. 008
1.0	5	3. 9± . 3	. 004± . 001	. 090± . 029
2.6	26	3. 8± . 2	. 004± . 001	. 098± . 015
4.0	4	4. 2± . 1	. 007± . 003	. 159± . 056

Table 6.—*Percentage fluoride in human bone ash from low-fluoride areas*

Fluoride level of water (ppm)	Bone	Number of samples	Fluoride in bone ash (percent)	Source of data
0.0	Rib	1	0. 01	Boissevain and Drea (*3*).
	Humerus	1	. 1	Do.
	Tibia	1	. 02	Do.
	"Toe"	1	. 16	Do.
Not given, presumably low.[1]	Skull	1	. 059	Klement (*19*).
	"Long bones"	(2)	. 070	Do.
	Rib	11	0. 048–. 21	Roholm (*1*).
0.1	Iliac crest	13	[3] . 028–. 128	Martin (*8*).
	Rib	83	. 006–. 252	Smith and associates (*7*).
	Vertebra	83	. 005–. 331	Do.
0.0–0.5	Rib	25	. 115–. 687	Glock and associates (*6*).
1.0	Iliac crest	20	. 034–. 132	Present study.
	Rib	30	. 033–. 207	Do.
	Vertebra	31	. 040–. 238	Do.
	Sternum	5	. 078–. 188	Do.

[1] Designated as "normal" individuals.
[2] Not given.
[3] Data originally given on dry, fat-free basis; calculated to ash basis assuming mean ash value of 59.0 percent for iliac crest from present study.

Table 7.—*Percentage fluoride in human bone ash from high-fluoride areas*

Fluoride level of water (ppm)	Bone	Number of samples	Fluoride in bone ash (percent)	Source of data
2.0	Rib	4	0. 12 –0. 35	Boissevain and Drea (*3*)
5.9 and 6.3	Miscellaneous	15	1. 47 –2. 21	Kilborn and associates (*5*)
4.4–12.0	Sternum	1	. 69	Linsman and McMurray (*4*).
	Vertebra	1	. 75	Do.
8.0	Miscellaneous	8	. 715– . 967	McClure and associates.[1]
1.0–4.0	Iliac crest	33	. 157– . 872	Present study.
	Rib	35	. 150– . 843	Do.
	Vertebra	36	. 208–1. 103	Do.

[1] This issue of *Public Health Reports*, pp. 741–746.

low nutritional status of this area (Chengtu, China).

Fluoride in the drinking water up to 4.0 p.p.m. did not affect the ash content of any of the bones analyzed.

For any given level of fluoride in the drinking water, the mean concentrations of fluoride in the dry, fat-free iliac crest, rib, and vertebra were similar. On an ash basis, however, the fluoride concentration of the vertebra was significantly higher than that of the rib at fluoride levels up to 2.6 p.p.m. At higher amounts of fluoride in the drinking water, this difference did not obtain.

Previous studies dealing with fluoride deposition in the white rat (*20–22*) and urinary fluoride excretion by the rat (*23*) and by man (*24, 25*) have indicated that as bone ages it may lose some of its capacity to store fluoride. This decrease in fluoride deposition with increasing age has been presented in graphic form by Hodge (*26*) based on the data on man of Smith and associates (7). In the present study, most of the subjects were of advanced age, and the means for the four groups varied only from 55.5 to 75.6 years. Hence, on the one hand, it was unlikely that a relation between fluoride deposition and age would be apparent; on the other hand, the age factor would tend not to bias any relation between bone fluoride and water fluoride.

From this extensive survey on the fluoride content of human skeletal tissues, it appears that the deposition of fluoride is directly related to the fluoride content of the drinking water up to 4.0 p.p.m. The deposition of fluoride in dentin and enamel is also elevated proportionately with an increasing concentration of fluoride in the drinking water (*27*).

There is no indication in these data or in those of previous studies that these human calcified tissues approach their theoretical capacity of about 3.5 percent fluoride, although drinking water containing as much as 4.0 p.p.m. fluoride was ingested. Prolonged exposure to the water of Colorado Springs, Colo., and Amarillo, Tex. (2.6 p.p.m. fluoride), and to that of Lubbock, Tex. (4.0 p.p.m. fluoride), elevated the fluoride content of the bone ash to a maximum of 1.080 and 1.103 percent, respectively.

The data from the five subjects of Grand Rapids, Mich., are of special interest. Exposure to water containing 1 p.p.m. fluoride did not exceed 12 years, and the average age of the subjects at the time they started to use fluoridated water was about 63 years. Nevertheless, a mean concentration of 0.146 percent fluoride on a dry, fat-free basis was found in these relatively old bones, compared with an average value of 0.049 percent in the bones of individuals of an average age of 56 years ingesting water containing less than 1.0 p.p.m. fluoride.

The fluoride data in the present study coupled with the microscopic findings on essentially the same individuals constitute substantial evidence that a concentration of fluoride as high as 0.548 percent in the dry, fat-free bone and 1.080 percent in the bone ash (for subjects in both studies) may be present without producing any apparent tissue damage.

SUMMARY

Selected bones and skeletal tissue—iliac crest, rib, vertebra, sternum, and intervertebral cartilage—obtained from 69 individuals 26 through 90 years of age who had drunk water containing 0.1 to 4.0 p.p.m. fluoride for at least 10 years were analyzed for ash and fluoride. These bones, which showed no significant histological changes, contained up to 0.548 percent fluoride on a dry, fat-free basis and 1.080 percent fluoride on an ash basis. The mean ash content of the iliac crest and ribs varied from 57.4 to 61.2 percent and that of the sternum and vertebra from 44.6 to 53.7 percent.

At any given level of fluoride in the drinking water, the various bones contained, on a dry, fat-free basis, similar concentrations of fluoride. The fluoride concentration of the intervertebral cartilage was considerably less than that of the bones.

The concentration of fluoride in the bones increased in an essentially linear fashion with an increase of fluoride in the drinking water up to 4.0 p.p.m.

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EXCESSIVE FLUORIDE IN WATER AND BONE CHEMISTRY
(Comparison of Two Cases)

F. J. McClure, H. G. McCann, and N. C. Leone

Chemical analyses of skeletal tissues of two women have provided new data on the effect on bone composition of excessive amounts of fluoride in drinking water. One of these women, subject A, 74 years old, lived for 24 years prior to death in Washington, D.C., where the drinking water contained 0.2 p.p.m. fluoride. The other, subject B, died at 78 years after 34 years of residence in Bartlett, Tex., where the drinking water contained 8.0 p.p.m. fluoride. Subject B, whose death was caused be a cerebral vascular accident, was a member of a population group previously studied (*1*). Subject A died of a heart attack.

The ingestion of fluoride and its concomitant occurrence in the animal body has been studied previously and extensively by the analysis of various tissues of cattle, sheep, swine, and small laboratory animals (*2–12*). Similar data for man, however, are limited and apply practically entirely to the bones and teeth.

Roholm (*2*) tabulated the results of the fluoride analyses of human tissues available in the literature up to 1937. His fluoride data, expressed as a percentage of the bone ash, are considered normal for the bones of adults without unusual exposure to the mineral. Values ranged from 0.05 to 0.21 percent fluoride.

He also reported the fluoride content of bones of two men who had been employed in the handling of cryolite and were thereby exposed to excessive quantities of the dust of this fluoride-containing mineral. The ash of bone specimens from these two cryolite workers contained as much as 1.31 percent fluoride.

Boissevain and Drea (*13*) reported 0.12–0.35 percent fluoride in ash of human rib bones after 15–40 years of exposure to a domestic water containing 2.5 p.p.m. fluoride. They found 0.07 percent fluoride in ash of one human rib with no known exposure to a fluoride drinking water. Linsman and Murray (*14*) attributed a case of so-called fluoride osteosclerosis to the use of drinking waters containing 4.4–12.0 p.p.m. fluoride. The ash of the sternum and that of lumbar vertebral body were reported to contain 0.69 and 0.75 percent fluoride respectively.

In 1938 Wolff and Kerr (*15*) analyzed the skeletal tissues of a man, 48 years of age, exposed almost daily for 18 years to the dust of rock phosphate (3.88 percent fluoride). According to their data, based on 9 different bone specimens, 0.18–0.70 percent fluoride was present in the dry, fat-free bone.

Kilborn and coworkers (*16*) observed not only a high incidence of mottled enamel in the inhabitants of a remote province of China but also a chronic skeletal disease which they attributed to fluorosis. The drinking water used in two villages contained 6.3 and 5.9 p.p.m. fluoride. A native of one of these villages, a 37-year-old man, was autopsied after accidental death. Sixteen different bones from this man averaged 1.27 percent fluoride in the dry bone and 1.91 percent fluoride in the bone ash. The results varied from 0.97 to 1.50 percent fluoride in dry bone and from 1.47 to 2.20 percent fluoride in bone ash.

The fluoride in the ribs of humans of different ages with no known abnormal exposure to fluoride was studied by Glock and associates (*17*). Although considerable variation occurred, there is evidence of an increase in skeletal fluoride concomitant with advancing age. Their results, which appear to be somewhat high, are:

Age (years)	*Percent fluoride in fat-free bone*	*Percent fluoride in bone ash*
Under 1	0. 024	--------
Under 1	. 033	--------
2	. 06	0. 115
15	. 088	. 174
22	. 18	. 397
25	. 061	--------
27	. 28	. 591
32	. 089	. 194
37	. 31	. 687
37	. 15	. 280
54	. 23	--------
59	. 09	. 186
60	. 23	. 473
68	. 18	. 380

This relation to age has been studied more recently by Smith and associates (*18*). In the age group 81–90 years, the fluoride in skeletal ash averaged a little more than 0.128 percent but ranged from 0.034 to 0.331 percent for 10 samples of rib and vertebra. Although marked variations thus occurred in the data for individuals of the same chronological age, these authors state that there is "a striking approximation to a linear relationship for the average bone fluoride concentrations when 10-year age groups are plotted against the logarithm of the age." These normal accumulations of skeletal fluoride were associated with the use of a drinking water containing 0.06 p.p.m. of fluoride.

In a more recent study, the fluoride content of the iliac crest was found to vary from 0.0164 to 0.0505 percent on dry, fat-free bones in persons aged 32–84 years who had no known exposure to a fluoride water (*19*). Extensive data on the relation between fluoride in drinking water and fluoride in skeletal tissues are reported by Zipkin and associates (see pp. 442-448 in this volume.

It is evident from these bone analyses that fluoride may accumulate in skeletal tissues concomitant with the ingestion of fluoride. The presence of relatively large quantities of fluoride in drinking water would be expected to cause marked increases in skeletal fluoride after a prolonged exposure. This was found to have occurred in the woman of this study exposed to high-level fluoride water.

While a major interest in the chemistry of these human skeletal tissues is the extent of fluoride accumulations, it was our purpose also to throw some light on the relation of fluoride to other bone elements, particularly those concerned with calcification. Accordingly, the ash, calcium, phosphorus, magnesium, and carbon dioxide contents of the bones were determined.

METHODS AND RESULTS OF ANALYSES

For the chemical analyses, the bones were reduced to small pieces; the fat was extracted with alcohol and ether; and the sample was ground to pass a 60-mesh sieve and then dried at 110° C. A temperature of 600° C. was maintained in the electric muffle furnace for ashing. Fluoride was determined on the ashed samples by standard procedures (*20, 21*), which require steam distillation using perchloric acid and titration of the fluoride evolved in the distillate with standard thorium nitrate. In addition to fluoride, calcium was determined on the ashed samples by triple precipitation of the oxalate from acid solution by slow addition of NH_4OH to avoid contamination with phosphate and to assure good separation of magnesium (*22*). Final weighing was as CaF_2. Magnesium was determined in the filtrate by double precipitation as magnesium ammonium phosphate. Phosphorus was determined on the unashed sample by the molybdovanadophosphate differential spectrophotometric method (*23*). Carbon dioxide was determined on the unashed sample by evolution with $HClO_4$ and absorption of the dried gas in a weighing bulb, a modification of the standard procedure (*22*).

In table 1 the analytical data are presented on the dry, fat-free bone. As might be expected, the most remarkable difference in the chemical content of the bones of these two aged adults lies in the fluoride. As much as 0.653 percent fluoride was present in the calvarium of the Bartlett woman, subject B, with a minimum of 0.512 percent fluoride present in the fibula. In striking contrast, the maximum percentage of fluoride in the skeletal tissues of the control, the nonfluoride subject A, was 0.092 percent in the calvarium. The fluoride concentrations in all the different dry, fat-free bone specimens were relatively constant.

Table 2 shows the percentage composition of the bone ash of the two women. It will be noted that the femur and fibula ash of B contained somewhat less fluoride, 0.771 percent and 0.715 percent respectively, than did the other specimens of bone ash of B, which were quite uniform and ranged from 0.855 to 0.966 percent fluoride. The femur ash of A also contained less fluoride than did the ash of other bones. One explanation of this observation may be the higher ash content of these bones.

Some differences in the ash, calcium, and perhaps phosphorus, in addition to fluoride, in the dry, fat-free bones of these two particular women occurred (table 1). In five possible comparisons of the same bones from these two similar individuals, more ash, calcium, and phosphorus were contained in the bone specimens of B than in those of A. These differences are most pronounced with respect to ash and calcium. There is a suggestion of some elevation in percentage of carbon dioxide in the bones of B compared with A. The calcium-phosphorus ratio and the magnesium content of the two subjects were comparable.

DISCUSSION

The results of the fluoride analyses of these human skeletal tissues agree with previous experimental and clinical evidence that skeletal tissues become an extensive depository of fluoride. The fluoride accumulations found in B resulted from the prolonged use of a drinking water containing an excessively high concentration of fluoride. As indicated by comparison of the data for these two subjects, this accumulation of fluoride increased slightly the calcification of skeletal tissue.

The ash and perhaps the calcium of dry, fat-free bones of B were slightly higher than the ash and calcium in normal human bones, as shown in table 3. Fluoride analyses of the bone specimens from normal subjects, previously studied by Illinois investigators (*24–26*), were made at the National Institute of

TABLE 1.—*Composition of skeletal tissues (dry, fat-free) of two adults with extremes of fluoride exposure*

Bone specimen	Percent ash		Percent calcium		Percent phosphorus		Calcium/phosphorus		Percent magnesium		Percent carbon dioxide		Percent fluoride	
	A[1]	B[2]	A	B	A	B	A	B	A	B	A	B	A	B
Femur	68. 73	70. 94	26. 46	26. 71	11. 70	11. 71	2. 26	2. 28	0. 37	0. 35	3. 93	3. 95	0. 062	0. 551
Tibia	65. 14	-----	24. 92	-----	11. 11	-----	2. 24	-----	. 38	-----	3. 44	-----	. 067	-----
Fibula	57. 43	71. 65	20. 92	26. 97	9. 24	11. 81	2. 26	2. 28	. 49	. 34	-----	4. 00	. 080	. 512
Calvarium	68. 10	71. 44	26. 12	26. 79	11. 61	11. 87	2. 25	2. 26	. 36	. 39	3. 70	3. 96	. 092	. 653
Lumbar vertebra	43. 39	60. 24	15. 69	22. 38	7. 46	9. 45	2. 10	2. 37	. 48	. 34	1. 48	3. 42	. 077	. 550
Thoracic vertebra	-----	60. 84	-----	22. 66	-----	10. 01	-----	2. 26	-----	. 33	-----	3. 41	-----	. 550
Dorsal vertebra	46. 38	-----	16. 72	-----	7. 50	-----	2. 33	-----	. 31	-----	2. 39	-----	. 086	-----
Miscellaneous vertebra	-----	58. 82	-----	21. 90	-----	9. 53	-----	2. 30	-----	. 33	-----	3. 15	-----	. 530
Rib	54. 78	66. 92	21. 10	25. 16	9. 50	11. 01	2. 22	2. 29	. 35	. 35	2. 92	3. 83	. 089	. 630
Right pelvis	-----	67. 53	-----	24. 56	-----	10. 79	-----	2. 28	-----	. 32	-----	3. 88	-----	. 577
Acromioclavicular joint	-----	55. 86	-----	20. 27	-----	8. 84	-----	2. 29	-----	. 32	-----	3. 19	-----	. 540
Crest of ilium	57. 77	-----	21. 90	-----	9. 81	-----	2. 23	-----	. 36	-----	2. 93	-----	. 078	-----
Intervertebral cartilage	6. 72	5. 19	1. 72	. 61	. 63	. 29	2. 73	2. 10	. 13	. 15	. 00	. 20	. 006	. 011

[1] Washington, D. C., resident; 0.2 ppm fluoride in drinking water.

[2] Bartlett, Tex., resident; 8.0 ppm fluoride in drinking water.

TABLE 2.—*Composition of bone ash of two adults with extremes of fluoride exposure*

Bone specimen	Percent calcium		Percent phosphorus		Calcium/ phosphorus		Percent magnesium		Percent carbon dioxide		Percent fluoride	
	A[1]	B[2]	A	B	A	B	A	B	A	B	A	B
Femur	38. 49	37. 65	17. 02	16. 51	2. 26	2. 28	0. 53	0. 49	5. 72	5. 57	0. 090	0. 771
Tibia	38. 25	------	17. 06	------	2. 24	------	. 58	------	5. 28	------	. 103	------
Fibula	36. 43	37. 64	16. 09	16. 48	2. 26	2. 28	. 85	. 47	------	5. 58	. 139	. 715
Calvarium	38. 36	37. 50	17. 05	16. 62	2. 25	2. 26	. 53	. 55	5. 43	5. 54	. 135	. 914
Lumbar vertebra	36. 16	37. 15	17. 19	15. 69	2. 10	2. 37	1. 11	. 56	3. 41	5. 68	. 177	. 913
Thoracic vertebra	------	37. 25	------	16. 45	------	2. 26	------	. 54	------	5. 60	------	. 904
Dorsal vertebra	36. 05	------	16. 17	------	2. 23	------	. 67	------	5. 15	------	. 185	------
Miscellaneous vertebra	------	37. 23	------	16. 20	------	2. 30	------	. 56	------	5. 36	------	. 901
Rib	38. 52	37. 60	17. 34	16. 45	2. 22	2. 29	. 64	. 52	5. 33	5. 72	. 162	. 941
Right pelvis	------	36. 37	------	15. 98	------	2. 28	------	. 47	------	5. 75	------	. 855
Acromioclavicular joint	------	36. 29	------	15. 83	------	2. 29	------	. 61	------	5. 71	------	. 966
Crest of ilium	37. 91	------	16. 98	------	2. 23	------	. 62	------	5. 07	------	. 135	------

[1] Washington, D. C., resident; 0.2 ppm fluoride in drinking water.

[2] Bartlett, Tex., resident; 8.0 ppm fluoride in drinking water.

TABLE 3.—*Comparison of composition of human skeletal tissue exposed to normal and high-fluoride water*

Subject and exposure	Dry, fat-free bone					Bone ash	
	Percent fluoride	Percent ash	Percent calcium	Percent phosphorus	Calcium/ phosphorus	Percent calcium	Percent phosphorus
Normal water fluoride:							
Male, 35 years[1]	----------	56. 67	21. 61	9. 48	2. 28	38. 13	16. 72
Male, 46 years[2]	0. 037	56. 89	22. 81	9. 86	2. 31	40. 09	17. 33
Male, 60 years[3]	. 038	56. 93	21. 26	10. 08	2. 11	37. 34	17. 70
Male, 48 years[3]	. 077	57. 85	22. 84	10. 30	2. 22	39. 48	17. 80
Female A, 74 years	. 079	57. 71	21. 72	9. 74	2. 23	37. 63	16. 87
Average of normal exposures	. 058	57. 21	22. 04	9. 89	2. 23	38. 53	17. 28
8.0 ppm water fluoride: Female B, 78 years	. 556	64. 91	24. 16	10. 55	2. 27	37. 22	16. 25

[1] Reference 24.   [2] Reference 25.   [3] Reference 26.

Dental Research. Analytical data for the individual bones of A and B have been averaged and are presented for comparison with the other findings.

There is a remarkably close agreement among the data obtained on the normal men and on our own normal subject A. Whereas the ash values of the bones from these subjects varied from 56.67 to 57.85 percent, the ash content of the bones of subject B averaged 64.91 percent. The calcium averaged 24.16 percent compared with a variation of 21.26 to 22.84 percent in adult bone exposed to low-fluoride water. There is no indication of an effect of fluoride on the calcium, phosphorus, and calcium-phosphorus ratio of the bone ash.

It seems evident, considering the data in table 3, that a slight increase in calcification did occur in the skeletal tissues of B. This is regarded as the result of exposure to excessive waterborne fluoride.

BONE PATHOLOGY AND FLUORIDE CONTENT

For previous evidence bearing on the relationship between fluoride content and pathological changes in human bone tissues, we must refer to the early classic studies of Roholm (*2*). The two autopsy cases he studied represent the characteristic skeletal pathology produced by excessive fluoride. The abnormalities were concomitant with 0.76 to 1.319 percent fluoride present in the ash of the affected bones. An unusually low value of 0.31 percent fluoride is reported for the frontal bone of one of these two cryolite workers.

The deleterious effects of fluoride, observed in experimental animal studies, have resulted consistently from excessive fluoride exposures, and the observations supply substantial information concerning the relationship between bone fluoride and bone pathology. Swine bones, according to Kick and associates (*3*), retained upwards of 0.30–0.40 percent fluoride before any toxic effects of fluoride were discernible. In the bones of dairy cattle suffering from extreme fluorosis, the severity of bone exostosis increased directly with the fluoride content, a mild form of exostosis being associated with 0.53 percent fluoride in the bone tissue (*9*).

Recently Suttie and associates (*27*) reported that dairy cows tolerated the ingestion of 30 p.p.m. of fluoride in the diet, with a concomitant accumulation of 0.46 percent fluoride in dry, fat-free rib bones. On the basis of the evidence that 0.01–0.15 percent fluorine is present normally in the bone ash of animals, Pierce (*6*) suggested that the "ingestion of quantities of fluorine which apparently exert no untoward effects on the general health of animals or which bring about no obvious morphological change in its skeleton, may nevertheless increase ten- to fifteen-fold the fluorine content of bones and teeth."

The data available through the present study provide additional evidence regarding the threshold level of fluoride which may be tolerated by human skeletal tissues. As much as 0.5–0.6 percent fluoride in the bones of B did not prove to be a physiological hazard. This is about 10 times the quantity of fluoride regarded as normal. X-ray examination, medical, and clinical studies made prior to the death of B (*1, 28*) do not reveal any skeletal abnormalities or systemic conditions of consequence to health or well-being which could be directly associated with the remarkable increases in the skeletal fluoride content. Other reports contain extensive information on the relation between fluoride content and bone pathology (see pp. 721–731 and 732–740 of this issue).

It must be concluded in the light of the available evidence that human skeletal tissue may have a very high degree of physiological tolerance to accumulations of fluoride.

SUMMARY

Analytic chemical studies of similar human skeletal tissues obtained at autopsy from two comparable women were conducted to determine the effect of a prolonged exposure to drinking water containing 8.0 p.p.m. of fluoride on the chemistry of human bones.

As a result of the prolonged use of this fluoride drinking water, the fluoride in dry, fat-free skeletal tissues ranged from 0.512 to 0.653 percent, as compared with 0.062 to 0.092 percent fluoride in the skeletal tissues of a subject, comparable in age, height, weight, and sex, with no unusual water-fluoride exposure.

There was some indication that the prolonged use of drinking water containing 8.0 p.p.m. fluoride accounted for an increase in the ash and a slight increase in the calcium content of the skeletal tissues.

The absence of any gross systemic findings, or of any impairment of health or well-being, malformation of the skeletal tissues, or malfunction generally in the one subject studied, indicates that human bone may not be affected by as much as 0.5 to 0.6 percent fluoride. These findings compare favorably with other previous evidence pertinent to human bone as well as fluorosed animal bones.

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PATHOLOGICAL STUDIES IN MAN AFTER PROLONGED INGESTION OF FLUORIDE IN DRINKING WATER

E. F. Geever, N. C. Leone, P. Geiser, and J. E. Lieberman

Public health programs to prevent dental caries by artificial fluoridation of drinking water have always considered the possibility of cumulative toxic effects. The present studies were instituted to determine whether pathological changes could be correlated with prolonged ingestion of fluoride-bearing water.

In an earlier publication we reported the necropsy findings in persons who had lived in a community with a fluoride level of 2.5 p.p.m. (*1*). This is 2½ times the level recommended for prevention of dental caries. Since necropsies do not routinely include microscopic examination of bones, a separate study of this phase of the question was undertaken. Persons coming to necropsy who had lived in communities with 1.0 to 4.0 p.p.m. fluoride, naturally occurring or added, in the drinking water were the subjects.

MATERIAL AND METHOD

Necropsies were performed in Grand Rapids, Mich. (1 p.p.m.), Colorado Springs, Col. (2.5 p.p.m.), Amarillo, Tex. (2.8 p.p.m.), and Lubbock, Tex. (4.0 p.p.m.). Bone specimens were obtained through the cooperation of local pathologists: Dr. Joseph Mann and Dr. Harold Bowman of Grand Rapids, Dr. Morgan Bethrong and Dr. Raoul Urich of Colorado Springs, Dr. Marie Shaw of Lubbock, and Dr. John Denko and Dr. C. P. Churchill of Amarillo. The series consisted of 37 persons who had resided for 10 years or more in the above communities. The majority, 24, were residents of Colorado Springs; they provided 65 bones for the study. The ages of the subjects, 17 men and 20 women, ranged from 36 through 90 years.

For comparison with the fluoride series, 33 persons, 24 men and 9 women, who had resided in essentially nonfluoride (less than 0.5 p.p.m.) communities served as controls. They ranged in age from 21 through 87 years.

Most of the persons in both series died suddenly. The most common causes of death were trauma, coronary heart disease, and cerebral vascular accidents. Those with chronic illness and diseases known to affect bone structure were excluded. Thus, there were none with primary or metastatic bone cancer, longstanding cancer of other organs, or renal or parathyroid disease.

In most cases three bones were selected for the microscopic studies: the body of a lumbar vertebra, a portion of iliac crest, and the sixth rib. In addition, the lumbar intervertebral body joints were examined for possible changes in articular cartilage. Less often, the sternum was also studied. A few bone or joint specimens proved unsatisfactory for some phase of the study, with the result that the totals vary.

The bones were fixed in 10 percent formalin and forwarded to the National Institute of Dental Research. Decalcification was carried out using a 5 percent formic acid method. Paraffin embedding and sectioning were performed and hematoxylin and eosin stains were applied, according to routine procedures.

Fluoride analyses of most of the specimens in both these series (plus a few other specimens) were performed independently. The results are reported in a separate article, by Zipkin and colleagues (pp. 442-448 in this volume).

PAROSTEAL AND MARGINAL TISSUE

Parosteal and marginal tissue consisted of laminated or loose collagenous elements often merging with and indistinguishable from periosteum. Striated muscle fibers, fat, blood vessels, lymph nodules, nerve trunks, or nerve ganglia were sometimes observed. In the sections of vertebra, the longitudinal ligaments were often included.

Focal, nonspecific, chronic inflammatory reactions were found in the marginal tissues of 4 bones from 3 persons in the control group. These foci were found adjacent to a lumbar vertebra in 3 bones and near the sternum in 1. Focal swelling and necrosis of striated muscle fibers were found in another control, a 59-year-old women who died of rheumatic heart disease. The changes, found near lumbar vertebra, were not accompanied by inflammatory reaction.

In the fluoride-exposed group, inflammatory change was observed in tissues of only one person. It consisted of a fairly severe subacute, nonspecific parostitis of the rib. In both the fluoride and the control groups small marrow foci were seen occasionally in periosteal and parosteal tissue of the vertebrae. These foci were undoubtedly related to the marrow of the vertebral body.

PERIOSTEUM

The periosteal surface was often irregular, microscopically, in both series. The irregularity in itself followed no consistent pattern. Sometimes slender, frayed or fuzzy papillary projections of bone and basophilic material, presumably calcium, were seen. Occasionally, smooth deposits of bone bulged up over wider areas under the periosteum. In other instances, new bone pushed up the periosteum in a trabecular pattern. Sometimes foci of bone were found in the parosteal tissue with no apparent connection to the subjacent bony cortex.

Irregularity in contour in some fields was due to old or active periosteal osteoclasia (figs. 1 and 2). Sometimes gaps were visible in the bone where blood vessels entered and the periosteum appeared to be the only barrier between the marrow and adjacent marginal tissue, particularly in the sections of vertebrae. The majority of the frayed or fuzzy papillary projections of bone and basophilic material appeared to be old and unaccompanied by cellular proliferation. However, small strips or foci of

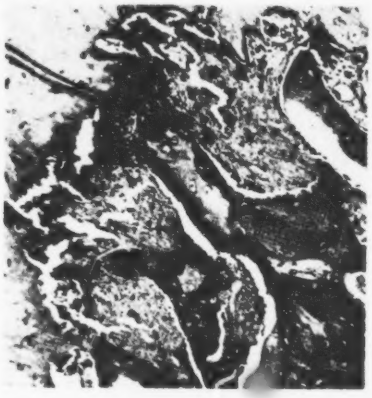

Figure 1.—*Active osteoclasia and fibrosis, periosteum of iliac crest, 105X, 44-year-old man, resident 40 years of a community with a natural fluoride level of 2.5 p.p.m.*

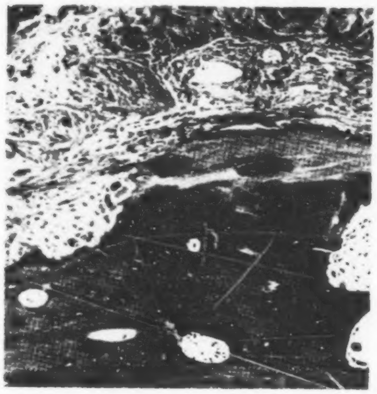

Figure 2.—*Active osteoclasia and fibrosis, periosteum and cortex of lumbar vertebra, 105X, 59-year-old man, control series.*

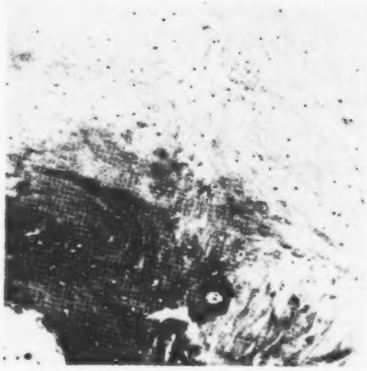

Figure 3.—*Parosteal and periosteal cartilaginous changes, iliac crest, 35X, 73-year-old woman, control series.*

bright, eosinophilic, new and incompletely calcified bone (osteoid) could be observed also. Often cuboidal or columnar osteoblasts were seen nearby.

Surface irregularity was frequently found near the sites of tendon insertion in the sections of iliac crest in both groups. Often it was accompanied by cartilaginous changes and irregular basophilic staining presumably due to calcification (fig. 3). Irregularity due to extension of cartilaginous and basophilic stained material along the front periosteal surface away from the intervertebral joint and toward the midlevel of the vertebral body was often seen.

The criteria used in grading excessive bone or calcium deposition were based on the maximum degree of projection from adjacent surfaces as measured by an ocular micrometer, the maximum foci per standard low-power microscopic field, the number of fields affected, and the presence of extraperiosteal bone or basophilic stained foci independent of the adjacent cortex. Bones from the fluoride and control groups were examined alternately.

Irregularity in periosteal contour due to excessive bone or calcium deposition, or both, was found in 48 of a total of 94 satisfactory bone sections in 25 of the 33 controls (table 1). Similar changes were present in 35 of a total of 99 satisfactory bone sections in 24 of 37 fluoride-exposed persons. Focal, nonspecific inflammation was found in 3 bones in 3 controls and in 2 bones in 2 of the fluoride group.

Table 1.—*Periosteal changes in persons ingesting fluoride and in controls*

Periosteal changes	Fluoride series		Control series	
	Number bones	Number persons	Number bones	Number persons
Total examined	99	37	94	33
Irregular periosteal bony proliferation and/or calcification	35	24	48	25
Focal periostitis	2	2	3	3
Excessive active osteoclasia	4	4	3	3

Active osteoclasia was found not infrequently with 1 or 2 osteoclasts recognizable in the margins of typical Howship's lacunae. Arbitrary criteria were set up to evaluate increased activity. Since the tissue sections were only approximately equal in size, quantitation could also be only approximate. Grading was started at +, for a mild, or slight, increase based on 2 to 4 multinucleated osteoclasts per low-power field associated with Howship's lacunae and present in more than one field. Grade + +, or moderate, was based on at least 5 osteoclasts, and grade + + +, or severe, on 7 or more osteoclasts per low-power field. On the basis of this scale, + + osteoclasia was observed in 2 bones and + in another for a total of 3 bones with such periosteal changes in 3 controls (table 1). In the fluoride group 2 bones showed a + increase, 1 a + +, and 1 a + + + increase in activity for a total of 4 bones in 4 individuals.

COMPACT CORTICAL BONE

Recently deposited bone (osteoid) could be determined by eosinophilic staining of the matrix, as mentioned above. This was particularly impressive when layers of various ages were arranged side by side. Configuration of cement lines, degree of cellularity, and appearance and arrangement of osteoblasts and osteocytes also aided. In the compact portion, swelling of endosteal cells was sometimes observed on surfaces abutting the marrow cavity, within the Haversian canals or within large open endosteal spaces. Sometimes such cellular arrangement was also associated with strips of bright eosinophilic new bone (osteoid). Other fields showed new bone layers without evident cellular activity. Serial section in such instances might have disclosed the presence of osteoblasts nearby. Some zones revealed what appeared to be mobilization and swelling of endosteal cells without evidence of new bone formation. This might have represented a preliminary phase in such a process.

The cortices of the rib and iliac crest were thicker than those of the vertebra, and the Haversian systems there were usually well defined. Sometimes, however, excessive decalcification made the lamellae appear delicate, poorly stained, and incompletely outlined.

An attempt was made to evaluate osteogenesis and osteoporosis objectively after the first survey of the material indicated that the differences in the amount of bone substance between the fluoride series and the controls would be slight, if there were any at all. No satisfactory method for such an evaluation could be found in the literature, and the following approach was therefore devised:

To obtain an overall impression of representative thickness, the compact layer was measured with an ocular micrometer, and the two extremes, for the thickest and the thinnest points were recorded. All measurements were made with the same microscope, 10X ocular, and the lowest power or scanning objective, 5X N.A. 14. A rough calibration

Table 2.—*Average coded measurements*[1] *for compact cortical bone, fluoride and control series*

Bone category	Fluoride series	Control series	Both series
All bones	1. 550 (99)	1. 734 (95)	1. 640 (194)
Lumbar vertebra along articular cartilage	1. 316 (34)	1. 418 (31)	1. 357 (65)
Lumbar vertebra cortex elsewhere	1. 510 (36)	1. 608 (31)	1. 556 (67)
Rib cortex	1. 893 (29)	2. 148 (33)	2. 029 (62)

[1] Square root of measurements.

Note: Figures in parentheses are numbers of measurements.

would be 1 oculomicrometer unit equals 0.1 mm. The iliac crest could not be measured satisfactorily because of marked irregularity of cortical contour. Hence only the measurements for the vertebra and the rib were used. Measurements of the cortex of the vertebral body adjacent to the hyaline articular cartilage were tabulated separately from those of the cortex of the vertebral body at other points, usually anterior and lateral. Osteoarthritic zones with spur or bridge formation were avoided as far as possible.

Statistical analysis of the measurements revealed no significant differences between the controls and the fluoride series (tables 2 and 3). The average coded measurements (square root of measurements) were 1.734 units for the controls and 1.550 for the fluoride series. The *F* value attributable to the difference was 3.74, and the theoretical *F* value with 1 and 57 degrees of freedom was 4.01 at the 5 percent probability level of significance.

Table 3.—*Analysis of variance, compact cortical bone measurements*

Source	d.f.	Sum of squares	Mean square	*F*	$F_{.05}$
Mean	1	521. 95			
Total	193	45. 85			
Bone part	2	[1] 14. 59	7. 29	[2]23. 52	3. 17
Control vs. fluoride	1	[1] 1. 16	1. 16	3. 74	4. 01
Sex	1	[1]. 01			
Interactions:					
Bone part × fluoride	2	[1]. 23			
Bone part × sex	2	. 77			
Sex × fluoride	1	. 02			
Within groups	57	17. 44	. 31		
Remainder	127	11. 64			

[1] Adjusted for unequal numbers.
[2] Significant at $P < .01$.

The mean measurements for the various bone categories (the lumbar vertebral cortex adjacent to the hyaline articular cartilage, the vertebral cortex elsewhere, and the rib cortex) did, however, differ significantly (tables 2 and 3). The *F* value attributable to differences in the measurements was 23.52, which is to be compared with the theoretical *F* value of 3.17. This comparison was independent of the fluoride-control comparison, and an adjustment was made for unequal numbers of bones in the three categories.

Abnormalities in bone architecture, such as irregular or excessive new bone deposition, resorption, excessive cellularity, fibrosis, and increased active osteoclasia were evaluated also. All bones were studied for these conditions.

Focal abnormality in cortical bone architecture was found in two bones in one control (table 4). Similar focal abnormality was observed in four bones in two of the fluoride group. An example of one type of abnormality observed, focal increased osteoid deposition, is shown in figure 4.

Active osteoclasia was believed increased slightly (+) in 10 bones, moderately (+ +) in 3 bones, and severely (+ + +) in 2 bones from 10 persons in the control series of 94 bones (fig. 5). In the fluoride group of 99 bones, osteoclasia was considered increased slightly (+) in 15 bones, moderately (+ +) in 4 bones, and severely (+ + +) in 1 bone from a total of 15 individuals.

Table 4.—*Cortical and medullary bone changes in persons ingesting fluoride and in controls*

Bone changes	Fluoride series		Control series	
	Number bones	Number persons	Number bones	Number persons
Total examined	99	37	94	33
Compact cortical bone				
Architectural abnormalities	4	2	2	1
Excessive active osteoclasia	20	15	15	10
Spongy medullary bone				
Architectural abnormalities	8	5	7	5
Excessive active osteoclasia	18	11	11	7

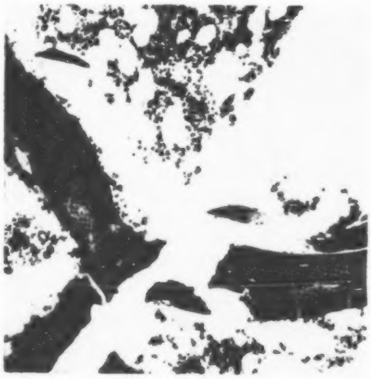

Figure 4.—*Increased deposition of osteoid tissue, rib trabeculae, 105X, 71-year-old man, resident 48 years of a community with a natural fluoride level of 4.0 p.p.m.*

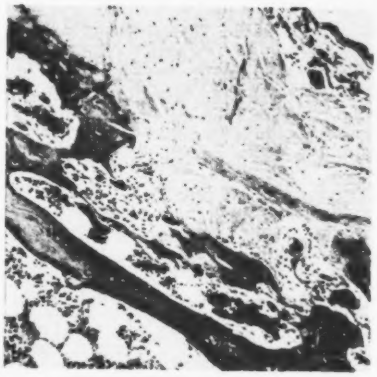

Figure 5.—*Increased active osteoclasia, vertebral cortex, 105X, 74-year-old man, control series.*

SPONGY BONE OF MEDULLA

An attempt was made, though admittedly the results are approximations only, to determine trabecular breadth or thickness by ocular micrometer measurement, as was done with the cortex. The two extremes were recorded, and a representative value was tabulated. Abnormalities in bone architecture and evidence of increased osteoclasia were recorded also.

The average coded measurements for the spongy medullary bone are given in table 5. The differences in values between the controls and the fluoride series were not significant when the figures were analyzed statistically. However, as in the cortical bone analysis, a statistical difference was found between the values for the three bone categories.

Table 5.—*Average coded measurements*[1] *for spongy medullary bone, fluoride and control series*

Bone category	Fluoride series	Control series	Both series
All parts	1. 134 (97)	1. 126 (90)	1. 130 (187)
Lumbar vertebra	1. 183 (36)	1. 185 (31)	1. 184 (67)
Iliac crest	1. 215 (32)	1. 264 (26)	1. 237 (58)
Rib	. 984 (29)	. 961 (33)	. 976 (62)

[1] Square root of measurements.

Note: Figures in parentheses are numbers of measurements.

Among the controls qualitative abnormalities were found in 7 bones in 5 persons (table 4). Focal architectural irregularity was seen in 6 bones, and a small osteoma, in the seventh (fig. 6). Similar focal irregularity in architecture was observed in 8 fluoride-exposed bones in 5 individuals. No tumors were found among the fluoride group.

Active osteoclasia was believed increased slightly (+) in 11 bones from 7 persons in the control series. In the fluoride group osteoclasia was considered increased slightly (+) in 14 bones, moderately (+ +) in 3, and severely (+ + +) in 1 bone from a total of 11 persons (fig. 7). In both groups active osteoclasia was often associated with osteoblastic activity and new bone deposition nearby. Variation in staining characteristics of the intercellular substance, configuration of the cement lines, degree of cellularity, and appearance and arrangement of osteocytes and osteoblasts, as mentioned in the discussion of the cortex, combined to provide fairly reliable impressions concerning relative ages of different bone layers.

BONE MARROW

Most marrow sections of both the fluoride and the control series showed active hematopoiesis. Of 93 satisfactory marrow sections in the control group, 63 were graded + + +; 25, + +; 4, +; and 1 was considered atrophic. Interesting qualitative features of the marrow sections from the controls included focal lymphoid hyperplasia (11 sections), focal erythroid hyperplasia (9 sections), and focal myeloid hyperplasia (7 sections). In 3 there was focal proliferation of megakaryocytes. The marrow of one bone revealed rather extensive fibrosis, and that of another showed focal hyperplasia of eosinphils. There were thus 32 bones with focal qualitative marrow changes.

Of 97 satisfactory marrow sections in the fluoride-exposed group, 69 were graded + + + quantitatively; 23; + +; and 5, +. Comparative qualitative study showed 17 with focal lymphoid hyperplasia, 1 with focal erythroid hyperplasia, and 4 with focal myeloid hyperplasia for a total of 22 qualitative changes.

INTERVERTEBRAL LUMBAR BODY JOINT

Osteophytosis occurred in both groups in the form of "spurs" or "lips," sometimes bridges (figs. 8 and 9). The osteophytes were composed of irregularly arranged osteoid tissue and mature bone, irregularly calcified cartilage, and fibrous tissue often associated with increased osteoclastic activity (fig. 10). The marrow in the osteophytes was usually fatty and deficient in hematopoietic elements. Accompanying the above process were mixtures of bone, fibrous tissue, and haphazardly calcified cartilage at and near the attachment of the osteophyte to the line of junction between hyaline articular cartilage and cortical body plate. The normally even contour along this line was usually disrupted. Varying degrees of thickening were noted in the subchondral bone along the line of attachment to the osteophyte. Large osteophytes were often associated with irregularity in appearance of that portion of the annulus fibrosus near the attachment of the osteophyte. Fibrillation, irregu-

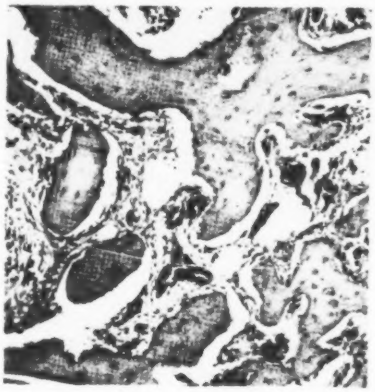

Figure 6.—*Small osteoma, rib medulla, 105X, 35-year-old man, control series.*

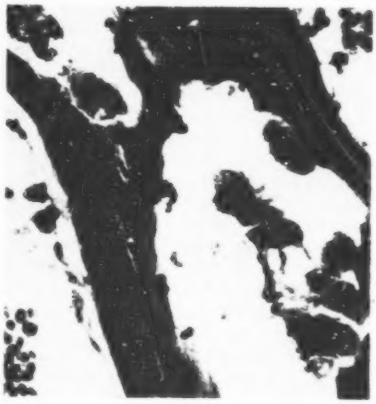

Figure 7.—*Increased active osteoclasia, trabecula of lumbar vertebra, 415X, 69-year-old woman, resident 28 years of a community with a natural fluoride level of 2.5 p.p.m.*

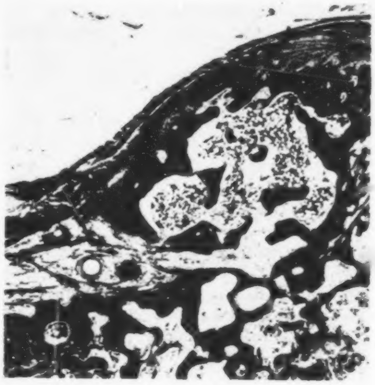

Figure 8.—*Osteophyte, lumbar vertebral body, 14X, 62-year-old woman, resident 19 years of a community with a natural fluoride level of 2.5 p.p.m.*

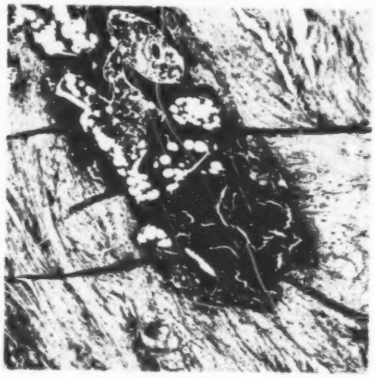

Figure 9.—*Fibrosis and increased osteoclasia in an osteophyte, lumbar vertebral body, 35X, 73-year-old woman, control series.*

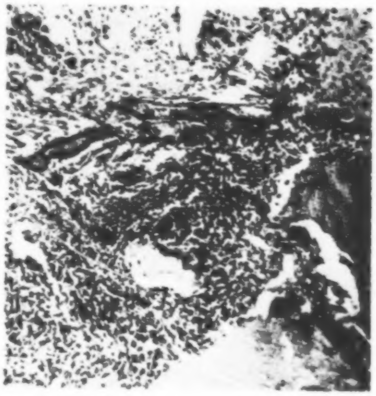

Figure 10.—*Increased active osteoclasia and fibrosis near osteophyte attachment, 105X, same bone and patient as in figure 9.*

larly increased porosity, or cystic degeneration was observed. Clefts in the intervertebral joint matrix were seen in both groups and were impossible to differentiate from artefacts.

The periosteum and anterior longitudinal ligaments over the osteophytic process revealed only nonspecific fibrous thickening.

No inflammatory reaction was noted within the annulus fibrosus or the nucleus pulposus even with advanced osteophytosis. The central portion of the nucleus pulposus was usually porous in the younger subjects (30–39 years) of both groups. In such cases multiple, irregularly shaped, cystic zones were apparent. They contained amorphous eosinophilic material in which occasional viable-appearing cartilage cells could be identified.

The annulus fibrosus in the young patients in both groups consisted of fairly dense layers of sparsely cellular fibrous tissue. As this tissue approximated the nucleus pulposus, it was arranged in interlacing bands among cartilage cells.

Also in the young patients the hyaline articular cartilage blended almost imperceptibly with the loose fibrocartilage or elastic cartilage of the nucleus pulposus and annulus fibrosus. Some variation in thickness was noted in the hyaline articular cartilage. The cartilage cells were arranged more regularly and their nuclei were oriented more nearly in parallel planes than those in the nucleus pulposus or annulus fibrosus. Occasionally, small clefts with loose connective tissue and capillaries penetrated the hyaline cartilage near its line of junction with the subchondral bone. The line of calcification was fairly even along this junction in the young patients, but prongs of cartilage extended beyond into the subchondral bone, and, similarly, microscopic prongs or tongues of bone and osteoid tissue projected upward into the hyaline articular cartilage. The marrow adjacent to the subchondral bone occasionally showed focal proliferation of delicate fibroblastic elements in the young patients of both groups.

In the aged patients of both groups cystic changes in the nucleus pulposus and fibrillar changes in the annulus fibrosus were more prominent. In the annulus near the periosteal junction, fibrosis and proliferation of vascular channels were sometimes observed. The vascular channels were occasionally arranged in clusters resembling telangiectasia.

Joint changes	Fluoride series	Control series
Total examined	31	35
Osteophytosis	12	18
Other evidence of osteoarthritis	4	5
Miscellaneous pathology	1	1

Table 6.—*Changes in lumbar intervertebral body joints*

Of 31 satisfactory sections of lumbar intervertebral joints from the controls, there were 12 with osteophytosis (table 6). The severity was rated arbitrarily from + to + + +. In 6 persons it was graded + + +, or severe. In the fluoride group of 35 satisfactory sections, there were 18 with osteophytosis. It was rated severe (+ + +) in 9. Other evidence of osteoarthritis included irregular proliferation of bone, osteoid tissue, cartilage, and fibrous elements disrupting the even line of junction of hyaline articular cartilage and subchondral bone. This irregularity was apart from that near the attachment of osteophytes described above. Such evidence of osteoarthritis was found in four controls and five of the fluoride group. Miscellaneous arthritic changes suggestive of trauma and accompanied by distortion of the joint were observed in one person in each series. There was no example in either series of rheumatoid spondylitis.

DISCUSSION

Reports of microscopic bone changes in man after chronic exposure to fluorides are rare. Furthermore, some of the cases reported have been complicated by chronic disease that could have contributed to the changes. Roholm (*2*) reported two cases 1 of which was complicated by a syphilitic infection. The other case occurred in a 68-year-old man exposed to cryolite for 25 years. The findings on gross and microscopic study included calcification of marginal tissues, ligaments, and tendons, increased thickening of the compact bone, irregular granular deposition of calcium in the intercellular substance, osteoid reaction around Haversian systems, and deposition of calcified fibrous tissue. The fluoride level of the bones was as high as 60 times normal values.

Another case, reported by Linsman and McMurray (*3*) and later listed in the Atlas of Orthopedic Pathology (*4*), was complicated by chronic renal disease. This occurred in a 22-year-old white man from a high-fluoride area in Texas. On microscopic examination his bones were described as having large trabeculae and "granular" architecture with lamellae which were irregular, not well seen, and with a tendency to fragmentation. Also described was some "condensation" of bone at the periphery of the trabeculae. The sternum contained 0.69 percent fluoride and the lumbar vertebra 0.75 percent.

In 1937 Bauer, Bishop, and Wolff (*5*) presented the findings in a worker exposed to phosphate rock containing about 4 percent fluoride. At necropsy the chemical concentration of the bones was 10 to 20 times normal fluoride values. The microscopic changes, reported only in a rib, included an increase in width of the cortex and thickening of the trabeculae. These investigators found no young osteoid tissue and no widespread deposits of calcium as described by Roholm. They observed little distortion of the normal bone architecture except reduction in the diameter of the Haversian canals and encroachment upon the marrow.

Weinmann and Sicher (*6*) advanced a tentative theory of the effects of fluorides in older persons and animals: first, there is a slow and incomplete destruction of compact bone and partial transformation into spongy bone; then, fairly dense osteophytes of both immature and mature bone and narrow osteoid seams are laid down as compensatory reinforcement, most prominent near the periosteum.

In our study microscopic changes in ligaments, parosteal tissue, and the periosteum were more numerous in the controls than in the fluoride series. Nothing comparable to Roholm's findings of calcification of marginal tissues, ligaments, and tendon was observed. Focal calcification and cartilaginous changes of the periosteum and adjacent tendon or fascia of the iliac crest were seen with equal frequency and intensity in both the control and the fluoride groups and were interpreted as normal.

Statistical analysis of measurements of bone mass in the cortex and medulla revealed no significant difference between the controls and the fluoride series. Since the fluoride group was approximately 12 years older than the control group (table 7), the differences in occurrence of osteoclasia and osteophytosis were studied further to determine whether there was any relation to age.

Ten of the 15 bones with increased active osteoclasia in the cortex of the

Table 7.—*Average age (in years) of persons supplying bone and joint specimens for microscopic examination*

Sex	Fluoride series	Control series	Both series
Both sexes	67. 8 (37)	55. 6 (33)	62. 0 (70)
Male	64. 5 (17)	54. 0 (24)	57. 3 (41)
Female	70. 5 (20)	60. 0 (9)	67. 2 (29)

Note: Figures in parentheses are numbers of persons controls were from 6 persons 60 years of age or over. There were 41 bones from 14 subjects in this age category; thus the incidence of increased osteoclasia, by bones, was 24.4 percent. By contrast 16 of the 20 bones with increased cortical osteoclasia in the fluoride series were from 16 persons 60 years of age or over. There were 68 bones from a total of 26 subjects in this age category for an incidence, by bones, of 23.5 percent.

Increased active osteoclasia in the medulla was similarly considered. Eight of the 11 bones of the controls with that finding were from 5 persons 60 years of age or over. Thus, the incidence for the 41 bones from this age category was 19.5 percent. In the fluoride series 17 of the 18 bones with that finding were from 10 persons aged 60 or over. The 68 bones from that age group yielded an incidence of 25.0 percent.

Examination of lumbar intervertebral body joints showed an increase in osteophytosis in the fluoride series. Many roentgenologists and orthopedists feel that osteophytosis in the lumbar vertebrae is not in itself to be considered a manifestation of arthritis or a cause for symptoms or disability (*7, 8*). Schmörl (*9*) found spinal osteophytes in 90 percent of all women over 60 years of age. On analyzing our data in the light of the difference in average age between the two groups, the difference in incidence of osteophytosis almost disappears. Nine of 13 patients, 69.2 percent, 60 years of age or over among the controls had osteophytosis as compared with 16 of 24, 66.0 percent, in the same age category in the fluoride series.

The differences in incidence of architectural abnormalities were not significant. Bone marrow examinations showed focal qualitative changes of minor importance. They were more frequent in the controls than in the fluoride series.

The results of this study can be correlated to some extent with previous work on necropsy material in Colorado Springs, where the fluoride level is 2.5 p.p.m. (*1*). In that study there was no significant difference in the occurrence of bone cancer between 334 long-term residents (more than 20 years) and 188 short-term residents (less than 5 years). There were 3 deaths attributed to bone cancer in the former group and 2 in the latter. The microscopic study of bones adds further support to the belief that fluoride in drinking water at a level of 1.0 p.p.m. can be ingested without cumulative toxic effect.

SUMMARY

Microscopic examinations were made of 99 bones from 37 persons coming to necropsy who had resided 10 years or more in communities where the drinking water contained 1 to 4 p.p.m. of naturally occurring or artificially added fluoride. Ninety-four bone specimens from 33 controls who had lived in areas where the drinking water contained less than 0.5 p.p.m. fluoride were used for comparison.

In addition to the bone specimens, the lumbar intervertebral body joints of the subjects were examined.

The microscopic examinations showed no significant differences between the fluoride-exposed group and the control group that could be related to fluoride intake. Microscopic changes in the bones and joints incidental to aging and due to nonfluoride-related conditions were observed in both series.

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RELATION OF THE FLUORIDE CONTENT OF HUMAN BONE TO ITS CHEMICAL COMPOSITION

I. Zipkin, F. J. McClure and W. A. Lee

ABSTRACT

A comprehensive study is presented on the composition of human bone over a wide range of skeletal fluoride concentrations. Thus, 69 samples of human bone, comprising iliac crest, rib and vertebra obtained from 23 individuals 26–90 years of age who had consumed drinking water containing up to 4.0 p.p.m. fluoride for 10–87 years prior to demise, were analyzed for calcium, phosphorus, magnesium, sodium, potassium, carbon dioxide and citrate. The percentage of calcium and phosphorus in the dry, fat-free bones was normal although they contained as much as 0.4 percent fluoride.

No relation was apparent between the fluoride present in bone ash over a tenfold range in concentration and its calcium or phosphorus content. With increased levels of fluoride in the ash (0.08–0.8 percent) there was a slight increase in magnesium and a decrease in carbon dioxide. The citrate content decreased markedly with increased fluoride. A slight decrease in sodium and little or no change in potassium were noted. The data support the hypothesis that fluoride is deposited in mature bone largely at the expense of surface limited ions.

In previous publications from this laboratory (Zipkin, McClure, Leone and Lee, 1958; McClure, McCann and Leone. 1958) data were presented demonstrating significant increases in the fluoride content of human bones as a result of a prolonged ingestion of fluoride in drinking water. Although above normal in fluoride content, no related changes in the bone tissues were apparent on histologic examination (Geever, Leone, Geiser and Liebermann, 1958). Previous information on the effect of increased fluoride on the chemical composition of human bones is quite limited. In one case reported by Wolff and Kerr (1938) excessive fluoride ingestion increased bone-ash fluoride up to 1.07 percent without, however, affecting the calcium, phosphorus or carbon dioxide content of the bones. Glock, Lowater and Murray (1941) and Call and Greenwood (1958) also reported no changes in ash, calcium and phosphorus in human bones containing up to 0.69 and 0.15 percent fluoride in the ash respectively. The former investigators also found no change in magnesium. McClure, McCann and Leone (1958) recently reported an increase in carbon dioxide, a reduction in magnesium and phosphorus but no change in the calcium content of the ash of a number of bones of one individual containing up to 0.97 percent fluoride.

Numerous experiments involving a variety of animal species have related the fluoride content of bone to a number of chemical constituents (McClure, 1939; McCann and Bullock, 1957; Weidmann, Wetherall and Whitehead, 1959). In these studies, unusually large quantities of fluoride ingested in food and water had produced relatively excessive deposits of bone-fluoride over a short space of time. While these experiments provide valuable information, the results are not generally applicable to conditions surrounding a prolonged human exposure to low levels of water-borne fluoride.

Continuing our studies on the effects of water-borne fluoride, the current report is concerned with the ash, calcium, phosphorus, sodium, potassium, magnesium, carbon dioxide, and citrate of human skeletal tissue in relation to its fluoride content. Analyses were made of 69 bone specimens from 23 individuals, selected from the previous studies (Zipkin et al., 1958; Geever et al., 1958) who were from 26 to 90 years of age and had consumed drinking water containing up to 4.0 p.p.m. fluoride for 10–87 years prior to death.

METHODS

Dry, fat-free bone samples were prepared as previously described (Zipkin et al., 1958) for the determination of carbon dioxide (Deakins and Burt, 1944) and citrate (Zipkin and McClure, 1949; Zipkin and Piez, 1950). The bone ash was analysed for calcium after double precipitation as the oxalate (Kolthoff and Sandell, 1952) to ensure good separation of magnesium. The precipitate was fired at 1000° C for one-half hour in a muffle, treated with hydrogen fluoride and evaporated to dryness on a steam bath, ignited to dull red heat over a bunsen flame and weighed as calcium fluoride. Phosphorus was determined on the ash (Gee and Dietz, 1953) and magnesium was determined on the filtrate from the calcium analysis by double precipitation as magnesium ammonium phosphate (Kolthoff and Sandell, 1952). Sodium and potassium were determined by internal standard flame photometry and interferences of calcium and phosphorus in the samples were overcome by adding similar amounts to the standards.

RESULTS

The quantity of fluoride in the bone ash varied tenfold, i.e., from 0.07 to 0.80 percent. A straight line function best describes the relation of the fluoride in the drinking water to that in the bone ash (Zipkin et al., 1958).

The bone analyses are presented on an ash basis in table 1 where the results are grouped according to the amount of fluoride in the individuals' drinking wa-

TABLE 1.—*Ash (dry, fat-free basis), fluoride, calcium, phosphorus, magnesium, sodium, potassium, citrate and carbonate content (ash basis) of selected human bones*

Bone sample	No.	F (%)	Ash (%)	Ca (%)	P (%)	Ca:P*	Mg (%)	Na (%)	K (%)	Na:K*	CO_2 (%)	Cit (%)
<1·0 p.p.m. F in drinking water												
Iliac crest	6	0·08±0·01	53·51±1·44	38·80±0·11	17·45±0·05	2·22±0·01	0·50±0·02	0·73±0·02	0·09±0·01	8·58±0·94	5·83±0·21	2·23±0·13
Rib	5	0·08±0·01	56·04±1·16	38·82±0·15	17·45±0·08	2·22±0·01	0·50±0·01	0·79±0·02	0·09±0·01	9·12±0·98	5·41±0·23	1·92±0·16
Vertebra	6	0·10±0·01	49·82±1·29	37·82±0·23	17·52±0·10	2·16±0·02	0·50±0·01	0·73±0·02	0·18±0·03	4·30±0·52	5·31±0·15	1·95±0·09
Sternum	5	0·13±0·02	48·59±1·95	38·39±0·21	18·05±0·22	2·14±0·02	0·46±0·01	0·67±0·03	0·06±0·01	13·77±2·62	4·67±0·10	1·86±0·16
1·0 p.p.m. F in drinking water												
Iliac crest	4	0·22±0·03	58·18±0·69	37·76±0·63	17·62±0·15	2·15±0·05	0·52±0·02	0·72±0·02	0·06±0·01	13·19±2·75	5·60±0·44	1·95±0·05
Rib	4	0·24±0·04	54·77±0·62	36·67±0·75	17·57±0·07	2·09±0·04	0·50±0·02	0·69±0·02	0·10±0·03	8·18±1·73	5·30±0·11	1·67±0·19
Vertebra	4	0·34±0·06	47·24±1·25	36·15±0·81	17·73±0·12	2·04±0·06	0·55±0·03	0·68±0·03	0·11±0·01	6·29±0·86	4·43±0·46	1·45±0·14
2·6 p.p.m. F in drinking water												
Iliac crest	7	0·40±0·09	58·92±0·61	38·12±0·33	17·05±0·11	2·24±0·02	0·58±0·02	0·72±0·03	0·08±0·01	9·50±1·38	5·08±0·18	1·75±0·11
Rib	7	0·41±0·09	57·47±1·16	38·08±0·32	17·33±0·11	2·20±0·02	0·57±0·02	0·69±0·02	0·07±0·01	10·06±1·20	5·08±0·24	1·64±0·07
Vertebra	8	0·56±0·11	49·79±0·75	37·24±0·40	17·59±0·23	2·12±0·02	0·63±0·02	0·72±0·03	0·17±0·02	4·44±0·44	4·24±0·23	1·36±0·08
4·0 p.p.m. F in drinking water												
Iliac crest	4	0·69±0·09	58·62±1·12	38·41±0·38	17·74±0·39	2·17±0·06	0·57±0·02	0·70±0·04	0·08±0·02	9·24±1·76	5·20±0·39	1·56±0·32
Rib	4	0·70±0·09	57·38±2·65	38·36±0·32	17·74±0·29	2·16±0·05	0·60±0·02	0·68±0·03	0·10±0·04	8·73±1·84	5·19±0·33	1·39±0·23
Vertebra	4	0·80±0·13	52·33±3·12	37·75±0·18	17·49±0·24	2·16±0·04	0·61±0·02	0·68±0·03	0·12±0·02	5·94±1·00	4·55±0·44	1·30±0·12

All estimates of error expressed as standard error.

* Based on means of individual ratios.

ter. The major bone constituents, calcium and phosphorus, in the bone ash and the calcium: phosphorus ratio remained unaffected by fluoride. A comparison of individual bones indicates that some slight increase in their ash content may be ascribed to fluoride. As the fluoride content of the drinking water increased from <1.0 up to 4.0 p.p.m., the ash of the iliac crest increased from 53.51 to 58.62 percent, of the rib from 56.04 to 57.38 percent and of the vertebra from 49.82 to 52.33 percent.

As the fluoride in the bones increased, magnsium increased in the ash of all bones from 0.50 percent to a maximum of 0.57, 0.60 and 0.61 percent for the iliac crest, rib and vertebra respectively. A slight but consistent decrease in sodium occurred in bones, i.e., in the iliac crest from 0.73 to 0.70 percent, in the rib from 0.79 to 0.63 percent and in the vertebra from 0.73 to 0.68 percent. In general the potassium concentration of the bone ash decreased slightly with increasing concentrations of fluoride.

With increasing fluoride the carbon dioxide decreased in all bones. Thus in the iliac crest the decrease was from 5.83 to 5.20 percent, in the rib from 5.41 to 5.19 percent and in the vertebra from 5.31 to 4.55 percent.

The most striking effect of the increase in bone fluoride was a 30 percent reduction of citrate in the bones of those individuals using water containing 4.0 p.p.m. fluoride. Thus, the citrate content of the ash of the iliac crest decreased from 2.23 to 1.56 percent of the rib from 1.92 to 1.39 percent and of the vertebra from 1.95 to 1.30 percent. The data on the citrate content of the bone expressed on an ash basis are shown graphically in fig. 1.

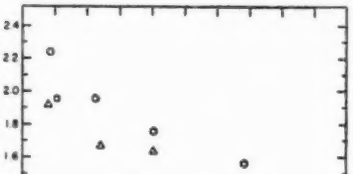

FIGURE 1.—*Relation of fluoride to citrate in bone expressed on an ash basis.* ○ *Iliac crest;* △ *Rib;* □ *Vertebra.*

DISCUSSION

A summary of our data on ash, calcium and phosphorus, along with other similar data for normal as well as highly fluorosed human bones, is presented in table 2. It is apparent that bones containing over 0.4 percent fluoride (0.8 percent in the ash) show no change in calcium and phosphate content. The increase in calcium and phosphorus in the dry, fat-free bones containing 0.6 percent fluoride is a reflection of the increased ash content which had a normal calcium but a slightly lower phosphorus content.

TABLE 2.—*Comparison of composition of human skeletal tissues exposed to normal (<1.0 p.p.m.) and fluoride waters*

Water-borne fluoride exposure	Dry, fat-free bone					Bone ash	
	Fluoride (%)	Ash (%)	Calcium (%)	Phosphorus (%)	Ca : P	Calcium (%)	Phosphorus (%)
normal*	0·045	58·20	21·70	9·95	2·18	37·29	17·09
normal†	0·058	57·21	22·04	9·89	2·23	38·53	17·28
<1·0 p.p.m.	0·049	52·82	29·36	9·23	2·20	38·48	17·47
1·0 p.p.m.	0·147	53·40	19·72	9·42	2·09	36·86	17·64
2·6 p.p.m.	0·247	55·40	20·97	9·62	2·19	37·81	17·32
4·0 p.p.m.	0·409	56·11	21·44	9·91	2·16	38·17	17·66
8·0 p.p.m.†	0·556	64·91	24·16	10·55	2·27	37·22	16·25

* CALL and GREENWOOD (1958).
† MCCLURE, MCCANN and LEONE (1958).

The present *in vivo* data confirm other *in vitro* findings that fluoride replaces carbon dioxide (Neuman et al., 1950), inhibits citrate uptake (Armstrong and Singer, 1956), and has no influence on calcium or phosphorus exchange (Neuman et al., 1950). An explanation for this result may reside in the fact that sodium, potassium, magnesium, carbon dioxide and citrate are presumed to be confined to the surfaces of the apatite bone crystals (Neuman and Neuman, 1953, 1958; Hendricks, 1952), although in these regards there may be some uncertainty concerning the position of magnesium (Klement, 1936) and carbon dioxide (McConnell, 1952; Buchanan and Nakao, 1958) in the crystal.

As regards magnesium, the apparent increase which accompanies fluoride deposition may be explained as due to its affinity for fluoride (McCann and Bullock, 1957). Thus the increase in magnesium would be secondary to fluoride deposition.

The observed reduction in citrate in these bones, concomitant with the increase in bone fluoride, suggests that citrate which is assigned to positions on the crystal surface, may be replaced by fluoride through ion-exchange processes.

Similarly it may be noted that due to cessation of bone growth, exchange reactions of bone surfaces would predominate in mature and aged bone. Advanced age at the time of initial exposure to fluoride thus favours the deposition of most of the fluoride at the expense of surface limited ions.

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EXPERIMENTAL LABORATORY RESEARCH RELATIVE TO THE EFFECTS OF FLUORIDE

Experimental Dental Fluorosis

A very sensitive manifestation of fluorosis in the white rat is the pronounced alteration in the development and appearance of the incisor teeth. This characteristic effect due to fluoride ingestion, is produced experimentally by adding fluoride to the diet or drinking water or by giving rats drinking water which is in use in an endemic mottled enamel area.

PUBLICATIONS

SEBRELL, W. H., DEAN, H. T., ELVOVE, E., and BREAUX, R. P.: Changes in the Teeth of White Rats Given Water from a Mottled Enamel Area, Compared with Those Produced by Water Containing Sodium Fluoride. Pub. Health Rep. *48*: 437–445, 1933.

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CHANGES IN THE TEETH OF WHITE RATS GIVEN WATER FROM A MOTTLED ENAMEL AREA COMPARED WITH THOSE PRODUCED BY WATER CONTAINING SODIUM FLUORIDE

By W. H. Sebrell, H. T. Dean, E. Elvove, and R. P. Breaux

INTRODUCTION

The manifestations of fluorosis in the incisor teeth of white rats were first described in detail by McCollum, Simmonds, Becker, and Bunting (*1*) although Schulz and Lamb (*2*), in an earlier publication, had noted an unusual overgrowth of the upper incisors of rats fed fluorine in the form of sodium fluoride. Sollman, Schettler, and Wetzel (*3*) studied the effect of sodium fluoride on albino rats by feeding graduated doses of 0.0002, 0.002, 0.01, 0.02, 0.04, and 0.23 percent of the ration. They report no effect on either growth or food consumption in the rats fed the three lowest amounts. They do, however, note a progressive impairment of growth and food consumption beginning with the 0.02 percent dose. These workers make no comment on changes in the teeth. McCollum and his associates (*1*) included 0.05 percent (500 p.p.m.) of sodium fluoride in an otherwise satisfactory stock diet. Eight of the ten animals of the first generation group were carried on the fluorine diet for over 200 days. These workers noted that the orange tint seen on the anterior surface of the incisors of normal rats was generally absent. The teeth grew abnormally, the superior incisors tending in nearly every case to curve backwards forming the arc of a circle and finally penetrating the roof of the mouth. This was presumably due to the fact that they were not worn down by attrition. McCollum and associates believe these changes to be due to alteration in structure and hardness. In 1927, Bergara (*4*) reported that white rats on 64 milligrams of sodium fluoride per kilogram of body weight incorporated into a diet of white bread and milk showed symmetrical coffee or chocolate colored bands on both the superior and inferior incisors after about 4 months' feeding. The bands are not unlike those described by Chaneles (*5*) and Pachaly (*6*). Tolle and Maynard (*7*), in 1929, reported that rats fed rock phosphate, treble superphosphate, and sodium fluoride showed the characteristic tooth changes described by McCollum.

The most complete report in the literature on the production of experimental fluorosis in white rats is probably that of Chaneles (*5*). This worker studied the effect of fluorine from several different angles. His experiments included feeding (*a*) fluorine as sodium fluoride, (*b*) sodium fluoride plus ultraviolet radiation, (*c*) sodium iodide, and (*d*) sodium fluoride and sodium iodide. The fluorine dosage was 50 milligrams of sodium fluoride per kilogram of body weight, and was incorporated into a diet of white bread and milk. The histopathology of the affected teeth is reported and illustrated in detail.

In an experiment conducted by McClure and Mitchell (*8*) groups of three paired white rats were fed approximately 0.02, 0.06, and 0.12 percent of sodium fluoride in the ration, and groups of four paired white rats were fed approximately 0.06 and 0.12 percent of calcium fluoride incorporated in the basal ration. They state that no visible effects were noted in the rats receiving the 0.02 percent of sodium fluoride, although higher levels of fluorine, both in the form of sodium fluoride and calcium fluoride, brought about changes in the teeth similar to those reported by McCollum and his associates (*1*). They further note that the insoluble calcium fluoride was as effective as the soluble sodium fluoride in bringing about changes in tooth structure.

The probable relationship between mottled enamel and the fluoride content of the drinking water was shown by Churchill (*9*). The first workers to associate experimental fluorosis in the white rat with the dental dystrophy known as mottled enamel were Smith, Lantz, and Smith (*10*). They report feeding rats St. David (an endemic mottled enamel area in Arizona) drinking water which had been concentrated by evaporation to one-tenth of the original volume. Seven samples of St. David water were analyzed for fluorine content and amounts varying from 3.8 to 7.1 parts per million were found. The amount of fluoride in the particular St. David water used was not stated. In another experiment they report incorporating St. David water residue directly into the ration fed the experimental animal. The amount so incorporated is not stated. At the end of a month the enamel of the teeth became dull, chalky white, and pitted. These same workers fed white rats sodium fluoride in graduated doses of 0.025, 0.05, and 0.1 percent of the ration and observed tooth changes similar to those reported by McCollum (*1*). They conclude that the tooth changes produced by feeding the St. David water concentrate and the residue from the St. David water are identical with the changes produced in the teeth by the inclusion of sodium fluoride in the ration.

Velu fed white rats calcium fluoride (*11*) and rock phosphate (*12*) containing about 3 to 4 percent of fluorine and reported the characteristic changes in the teeth described by previous workers. He (*13*) also reported that a similar condition was produced by feeding groups of white rats water associated with Moroccan and Algerian rock phosphate.

Smyth and Smyth (*14*) report tooth changes similar to those described by McCollum and his associates (*1*) in white rats fed fluorine in the form of cryolite (Na_3AlF_6) and barium fluosilicate ($BaSiF_6$). The fluorine compound was mixed with the basic diet. Bethke, Kick, Hill, and Chase (*15*) state that fluorine added to the diet of rats results in a hypoplasia of the enamel and dentine and that the severity of the hypoplasia is proportional to the amount of fluorine ingested and the form in which it is added to the diet. In their experiments they utilized four different forms of fluorine compounds in dosages of equivalent fluorine content and found that calcium fluoride produced the least and sodium fluoride the greatest deleterious effect on the teeth. Rock phosphate and phosphatic limestone both produced an effect intermediate between that of calcium fluoride and sodium fluoride.

EXPERIMENTAL

The purpose of this experiment was to compare the changes in the teeth of white rats receiving small quantities of sodium fluoride in the drinking water with those produced by water from an endemic mottled enamel area. Conway, Horry County, S.C., is an endemic mottled enamel area of particular interest to us since a survey by Dean (*16*) has shown a high incidence of mottled enamel among the children using the municipal water supply during the period of calcification of their permanent teeth.

Water from the municipal water supply was shipped in 5-gallon Pyrex glass bottles to this laboratory for the experiment. A sample of this water was analyzed by the methods given in the Standard Methods of Water Analysis of the American Public Health Association. The fluoride was estimated by the ferric chloride method, using a procedure similar to that used by Churchill (9). Controls were carried out with synthetic waters, which were prepared on the basis of the chemical analysis, but omitting the fluoride or adding known quantities of it. The results obtained are given in table 1.

TABLE 1.—*Analysis of municipal water of Conway, S.C.*

	Parts per million
Residue on evaporation (180° C.)	640.0
Loss on ignition	17.5
Fixed residue	622.5
Silica (SiO_2)	19.0
Iron (Fe)	.04
Calcium (Ca)	1.9
Magnesium (Mg)	.9
Sodium (Na)	250.3
Potassium (K)	5.1
Carbonate (CO_3)	20.4
Bicarbonate (HCO_3)	528.9
Sulphate (SO_4)	5.6
Nitrate (NO_3)	.4
Chloride (Cl)	48.5
Fluoride (F)	6.0

Since the etiological factor in the production of mottled enamel is apparently associated with the water supply it was decided in this experiment to incorporate the fluoride into the drinking water. The experimental animals were divided into five groups of six rats each. All received the same diet, the composition of which is given in table 2. It is to be noted that the salt mixture used in this diet adds about 10 parts per million of fluorine to the diet.

TABLE 2.—*Composition of diet*

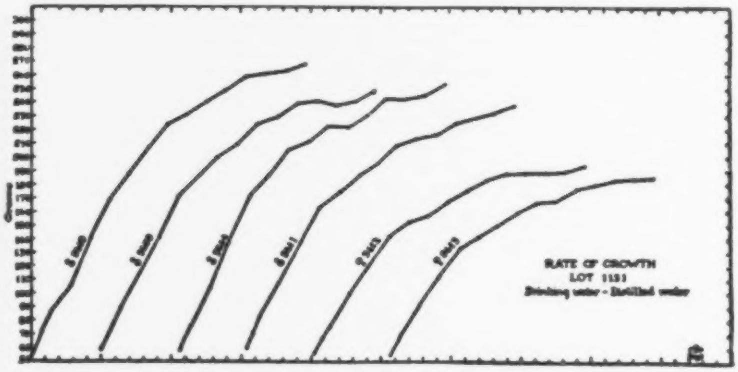
	Percent
Casein, leached	20
Salt mixture	4
Brewer's yeast	5
Cottonseed oil	3
Cod-liver oil	2
Cornstarch	66

The rats were kept in individual metal cages having screen bottoms of ¾-inch wire mesh. The diet was weighed and served in glass cups. About every three days the residue was weighed and the cups refilled. The drinking water was supplied in rubber-stoppered glass bottles attached to the outside of the cages, the rats having access to the water by means of glass tubes. An attempt was made to check the amount of water consumed by measuring the residue, but no accurate estimate could be made because of the inability to measure the quantity of water wasted, which in some instances represented a considerable amount.

The rats were examined carefully three times a week, and weighed once a week. The pellets of feces were counted and the consistency was noted three times a week. No evidence of diarrhea was seen in any of the exprimental animals.

Group I (lot 1131) was given distilled drinking water. These animals grew normally and presented no evidence of disease. The teeth showed the orange color of normal rats (fig. 1). The rate of growth is shown in chart 1.

CHART 1



Group II (lot 1130) was given Conway, S.C., water which had been concentrated to one-tenth of its volume by evaporating at about 85°–90° C. These animals grew normally and the only gross pathological changes noted were in the teeth. Within 10 days from the beginning of the experiment the normal orange color had disappeared from the labial surfaces of the lower incisors, which appeared whitish except for an opaque orange spot at the tip of each tooth. Within the next week the lower incisors became a translucent white throughout their length. Within 52 days small brown spots appeared on the labial surfaces of the lower incisors (fig. 2). Within 80 days these brown spots had covered the entire length of the lower incisors and assumed the appearance of closely spaced, narrow, brown, transverse striations. The rate of growth is shown in chart 2.

Group III (lot 1129) was given a synthetic drinking water comparable quantitatively to the concentrated Conway water and containing all of the ions shown by the Conway water analysis to be present in amounts greater than one-half of one part per million, with the exception of fluorine. This lot was carried in order to determine whether this mixture of mineral salts without fluorine would cause any recognizable tooth changes. These animals grew normally and presented no evidence of disease. The teeth showed the orange color of normal rats. The rate of growth is shown in chart 3.

Group IV (lot 1132) was given a synthetic drinking water containing 150 parts per million of sodium floride in distilled water. These animals grew normally and the only gross pathological changes noted were in the teeth. The rate of growth is shown in chart 4. The teeth showed changes similar to those appearing in the teeth of the rats on

CHART 2

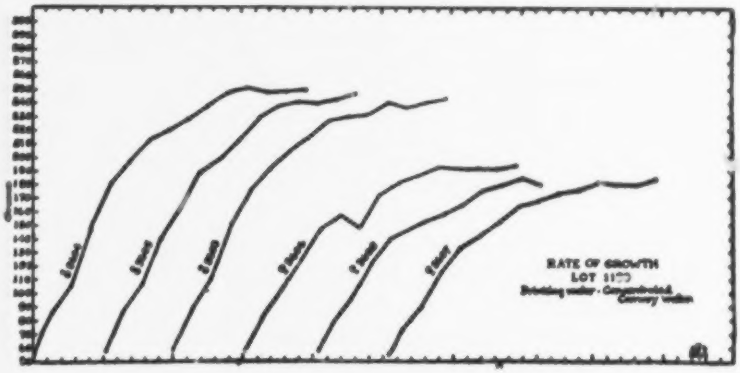


CHART 3

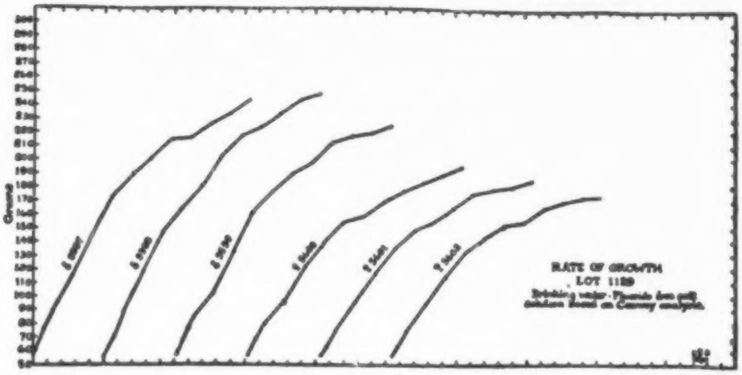


CHART 4

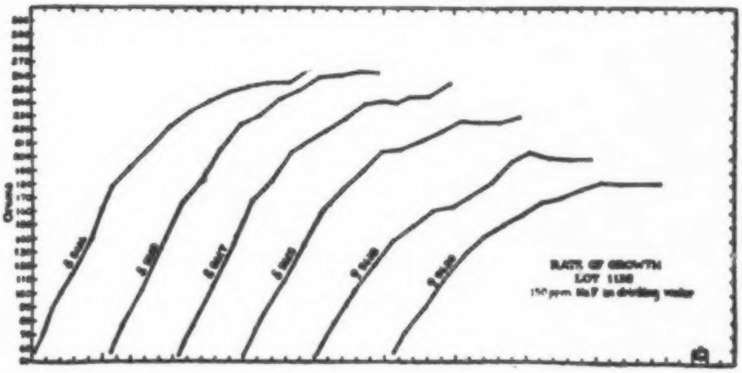
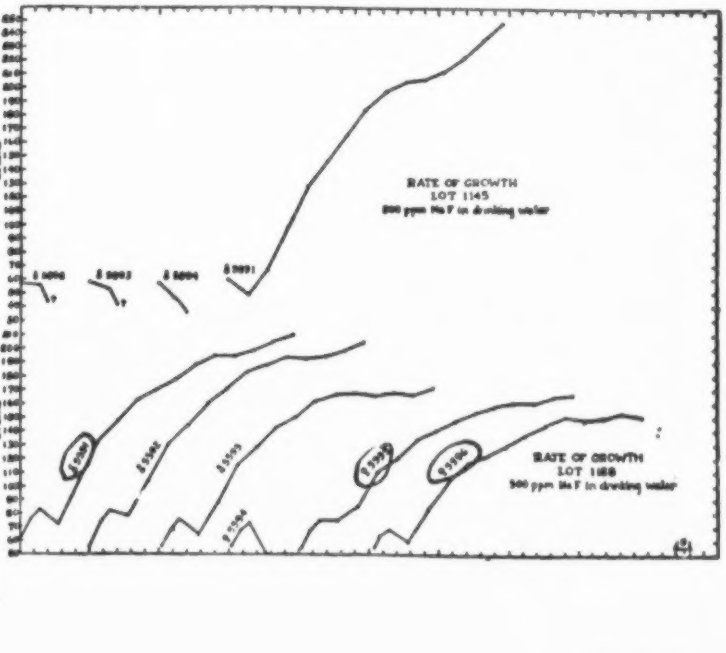


CHART 5



Conway water. Within 10 days from the beginning of the experiment the normal orange color had disappeared from the labial surfaces of the lower incisors, which appeared whitish except for an opaque orange spot at the tip of each tooth. Within the next week the lower incisors became a translucent white throughout their length. Within 52 days from the beginning of the experiment, small brown spots appeared on the labial surfaces of the lower incisors. These spots increased in size and number until they were scattered over the entire labial surfaces. They appeared to differ from the lesions noted in the teeth of the rats on the Conway water only in their more irregular distribution.

Group V (lot 1128) was given distilled water containing 500 parts per million of sodium fluoride. The animals on this water gained weight for the first three days and then lost weight during the next week, followed by a rapid and continuous gain in weight, with the exception of one rat which died in 11 days from the beginning of the experiment. The remaining animals showed no gross pathological changes except in the teeth. The rate of growth is shown in chart 5. Within 10 days the lower incisors had lost their normal orange color and were blanched except for an opaque orange spot at the incisal tip. The teeth then gradually became chalky white and brittle. By the end of 52 days the teeth of some of the rats had broken off at the gingival margin or at the tips so that the opposing teeth, apparently because of lack of wear, became abnormally long. The upper incisors then tended to form the arc of a circle and penetrate the palate.

An attempt was made to repeat the latter part of the experiment (i.e., with 500 p.p.m. of NaF) using a total of 20 rats. Sixteen rats died within 11 days. The four that survived the acute toxic effects of the fluorine grew well but showed the same chalky white, brittle teeth that were seen in the first group on this water. The rate of growth of one lot (lot 1145) is shown in chart 5.

SUMMARY

A drinking water from an endemic mottled enamel area (Conway, S.C.) concentrated to one-tenth of its volume produced whitish incisors in white rats followed by the appearance of brown striations.

A synthetic drinking water comparable to the concentrated Conway water, and containing all of the ions found in the Conway water in amounts greater than one-half of one part per million except-

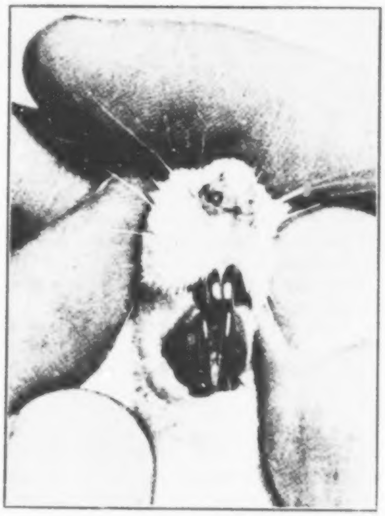


Figure 1.—*Teeth of rat receiving distilled drinking water, showing appearance of incisors of normal rat. The apparent white spots are due to high lights.*

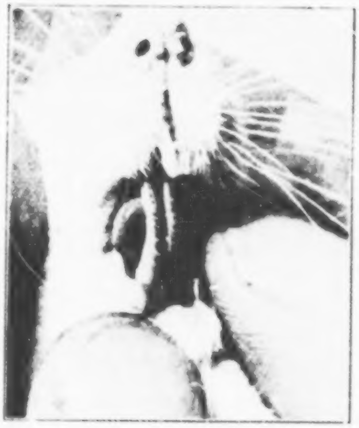


Figure 2.—*Teeth of rat receiving concentrated Conway water, showing loss of normal color and appearance of brown spots on incisors.*

ing fluorine, did not cause any noticeable abnormality in the teeth of white rats.

A synthetic drinking water containing 150 parts per million of sodium fluoride caused a loss of the normal orange color of the incisors of white rats followed by the appearance of irregular brown spots similar to the changes produced by the Conway water.

A synthetic drinking water containing 500 parts per million of sodium fluoride was exceedingly toxic to young white rats and produced chalky white, brittle teeth in those surviving the acute toxic effect.

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EFFECT OF VARIOUS AMOUNTS OF SODIUM FLUORIDE ON THE TEETH OF WHITE RATS

By H. TRENDLEY DEAN, W. H. SEBRELL, R. P. BREAUX, and E. ELVOVE

In a previous publication (*1*), the present authors reviewed the literature on the effect of fluorine on the teeth of white rats, and both DeEds (*2*) and McClure (*3*) have recently made extensive reviews of the literature on the toxic effects of fluorine. It seems unnecessary, therefore, at this time to refer again in detail to the literature.

In our previous report, the changes produced in rats' teeth by 150 p.p.m. and 500 p.p.m. of sodium fluoride in the drinking water were described. The purpose of the present experiments was to determine the smallest quantity of sodium fluoride in the drinking water which would produce visible changes in the teeth of white rats under similar conditions, and to observe the changes in the teeth produced by various amounts of fluorine. Accordingly, a group of young rats (55 to 60 grams in weight) was divided into six lots. The rats were placed in individual metal cages having screen bottoms and were fed our diet 411, the composition of which is given in table 1.

The amounts of sodium fluoride in the drinking water and the results are summarized in table 2.

The group (lot 1156) receiving the smallest amount of sodium fluoride in the drinking water (50 p.p.m.) began to show changes in the teeth 28 days from the beginning of the experiment. At this time, very fine, closely spaced, brown striations could be made out with a hand lens on the gingival third of the lower incisors. These striations were so fine that they could not be seen with the naked eye, except with the very best illumination. The striations involved progressively the entire labial surface of the lower incisors, but were so fine and so closely spaced that they were visible only on careful observation. The four rats in each of the groups (lots 1144 and 1143) which were given 75 p.p.m. and 100 p.p.m. of sodium fluoride in the drinking water showed the brown striations, which had covered the entire labial surface of the incisors within 56 days from the beginning of the experiment. These striations were more readily seen than those in the rats given 50 p.p.m. and tended to become irregular patches similar to those seen in the rats receiving 150 p.p.m. of sodium fluoride, but in a lesser degree.

The results with 150 p.p.m. (lot 1147) confirm our previous results. Twenty-one days from the beginning of the experiment it was noted that the lower incisors were somewhat lighter in color than the teeth of the control rats, and by the 28th day fine brown striations were visible on the gingival third of the lower incisors, which extended to and covered the entire labial surface of the teeth by the 35th day, and the color of the teeth had changed from the normal orange-brown to a cream color. The fine, brown, concentric striations gradually became larger and irregular, and the teeth were finally covered by irregular brown patches.

In our previous experiments (*1*) it was noted that, of the rats given drinking water containing 500 p.p.m. of sodium fluoride, a number died, and the survivors had white, chalky, brittle incisors which fractured readily and did not show any of the brown striations noted in the group given 150 p.p.m. of sodium fluoride. Therefore, a group (lot 1163) was given a drinking water containing 300 p.p.m. of sodium fluoride to determine the effect of an intermediate quantity. On this dosage, none of the experimental animals died. Ten days from the beginning of the experiment the lower incisors of all of these rats showed beginning loss of pigment on the gingival third, which followed progressively the

TABLE 1.—*Composition of diet 411*

Ingredients	Percent by weight	Ingredients	Percent by weight
Casein, leached	20	Dried brewers' yeast	5
Cottonseed oil	3	Corn starch	66
Cod-liver oil	2	Salt mixture (O. & M.)[1]	4

[1] Prepared according to Osborne and Mendel, Jour. Biol. Chem., vol. 37, p. 572 (1919).

TABLE 2.—*Changes in the teeth of rats on diet 411 with various amounts of sodium fluoride in the drinking water*

Lot no.	Number of rats	Amount of NaF in drinking water (p.p.m.)	Changes in labial surface of lower incisors
1146	4	None	None.
1156	4	50	Very fine brown striations; seen best with hand lens.
1144	4	75	Fine brown striations.
1143	4	100	Do.
1147	2	150	Brown striations later changing to irregular brown patches.
1163	4	300	Loss of pigment; brittle; no brown striations.

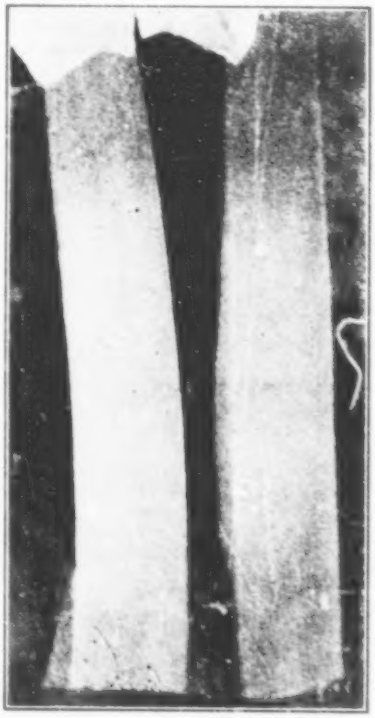


FIGURE 1.—*Normal lower incisors of control rat 5696, lot 1146, on diet 411 with distilled drinking water.*

tooth growth, and within 20 days the entire labial surface of the incisors was yellowish-white in color. Within 45 days the lower incisors of all rats showed fractured tips. There was no evidence of the brown striations seen with the smaller quantities of fluorine, nor did the teeth appear to be as chalky and brittle as those of the rats receiving 500 p.p.m. of sodium fluoride.

When it was seen that as little as 50 p.p.m. of sodium fluoride produced gross changes in the teeth, it was felt that the small amount of sodium fluoride used in the preparation of the Osborne and Mendel salt mixture (approximately 10 p.p.m. of fluorine in the diet) might be influencing the results. Therefore, a new series of young white rats (weighing 55–60 grams) were fed diet 411–A, which is identical with diet 411 (table 1) except that no sodium fluoride was used in the preparation of the Osborne-Mendel salt mixture. In addition to a control lot on distilled water, three other lots of rats were given drinking water containing 25, 50, and 500 p.p.m. of sodium fluoride, respectively. The results are summarized in table 3.

Table 3.—*Changes in the teeth of rats on diet 411–A*[1] *with various amounts of sodium fluoride in the drinking water*

Lot no.	Number of rats	Amount of NaF in drinking water (p.p.m.)	Changes in labial surface of lower incisors
1181	4	None.	None.
1184	4	25	Exceedingly fine brown striations clearly visible only with hand lens.
1182	4	50	Very fine brown striations best seen with hand lens.
1199	4	500	White, chalky, brittle hypoplastic enamel.

[1] No sodium fluoride in Osborne-Mendel salt mixture.

Changes in the teeth of the rats (lot 1184) receiving 25 p.p.m. of sodium fluoride in the drinking water were first noted 23 days from the beginning of the experiment. In the sunlight, with the aid of a hand lens, exceedingly minute, brown striations could be made out on the labial surface of the lower incisors. No such changes could be seen at any time on the teeth of the control rats (lot 1181) on distilled water.

The rats (lots 1182 and 1199) on 50 p.p.m. and 500 p.p.m. of sodium fluoride in the drinking water showed changes in the teeth similar to the changes previously noted in rats on diet 411 with the same amount of sodium fluoride in the drinking water.

FLUOROSIS AND CALCIUM METABOLISM

The possibility that fluorosis may bear a relationship to calcium metabolism may be implied from the work of Bergara (*4*), Chaneles (*5*), Smith, Lantz, and Smith (*6*), McClure and Mitchell (*7*), and indirectly by Pierle (*8*). DeEds (*2*) states

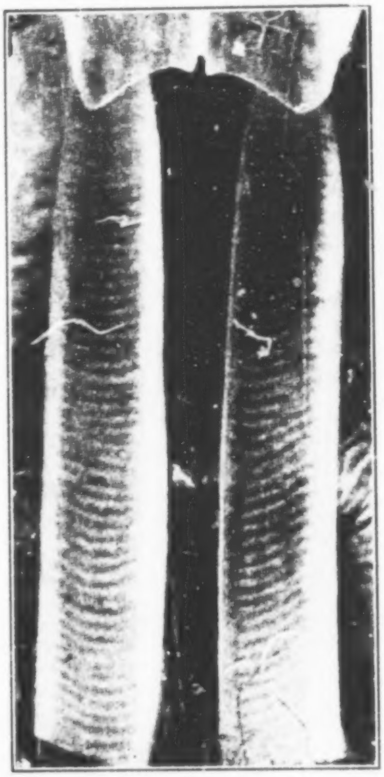


Figure 2.—*Lower incisors of rat 5954, lot 1156, on diet 411 with 50 p.p.m. NaF in drinking water. Teeth show fine, regular, brown striations.*

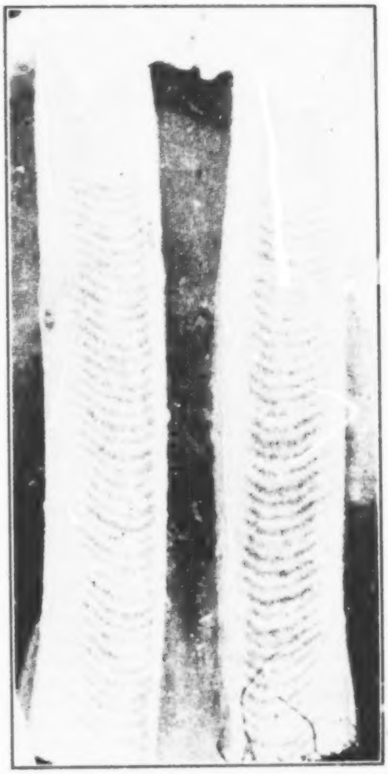


Figure 3.—*Lower incisors of rat 5683, lot 1144, on diet 411 with 75 p.p.m. NaF in drinking water. Fine, brown striations slightly more prominent than those shown in fig. 2.*

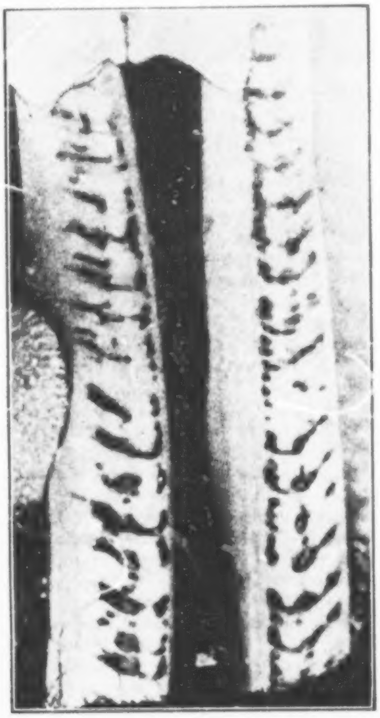


Figure 4.—*Lower incisors of rat 5899, lot 1147, on diet 411 with 150 p.p.m. NaF in drinking water. Teeth white; brown striations broken and irregular.*

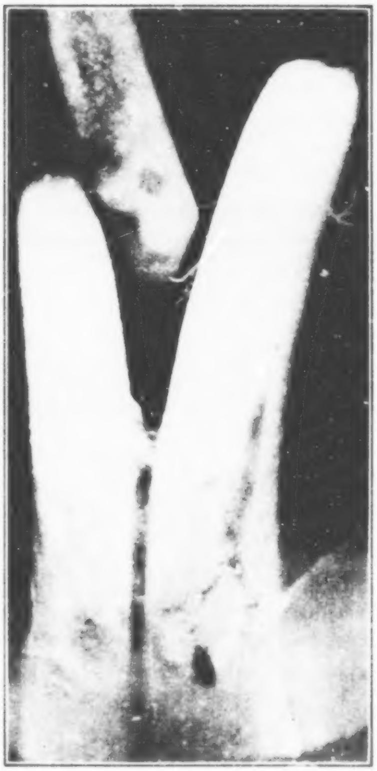


FIGURE 5.—*Lower incisors of rat 6201, lot 1199, on diet 411-A with 500 p.p.m. NaF in drinking water. Teeth white; tips broken; enamel fractured; no evidence of brown striations.*

that "a low calcium intake hastens the onset and development of chronic fluorine intoxication, whereas a liberal calcium intake tends to offset the effects of fluorine * * *." McClure and Mitchell (*7*) concluded that "the calcium balance of the rats was not affected by either sodium fluoride or calcium fluoride at levels of 0.0106 percent and 0.0313 percent fluorine in the ration. The results indicate, with a probability equal to a practical certainty, that a level of 0.0623 percent of fluorine in the form of either calcium fluoride or sodium fluoride, lowered the percentage of calcium that otherwise would have been retained by the experimental rats."

Smith and Lantz (*9*) state that "when sodium fluoride was incorporated in the ration at the level of 0.05 percent, the analyses of teeth and bones showed no significant alterations in the percentage of ash, calcium, or phosphorus, or in the calcium to phosphorus ratio. When the concentration of sodium fluoride was increased to 0.1 percent of the ration, the teeth and bones were lower in ash content but contained a greater percentage of calcium, lower percentage of phosphorus, with a higher calcium to phosphorus ratio."

Hauck, Steenbock, and Parsons (*10*), (*11*), in a series of experiments in fluorine toxicosis with 1,500 p.p.m., of sodium fluoride incorporated into varying diets, conclude that "it is evident that the Ca content of the diet is an important factor in determining the effect on body growth, maintenance, and the ash content of the bone and teeth."

Such changes in calcium balance, however, as have been noted by these investigators (*7*), (*9*), (*10*), (*11*) were observed in experiments wherein the fluoride dosage was in excess of the maximum amount used in our series.

The pigmented incisors of the white rat are very sensitive indicators of fluorosis. Our experiments indicate that a dosage as low as 25 p.p.m. of sodium fluoride in the drinking water results in a definite sign; namely, exceedingly fine, brown striations. With this in view, a series of four lots of rats were placed on diet 411-B, which is identical with diet 411 except that 5 percent calcium carbonate is added and the corn starch correspondingly reduced. Three of the lots (1193, 1192, and 1185) were given 50, 150, and 500 p.p.m. of sodium fluoride, respectively, in the drinking water; one lot (1191) was given distilled drinking water. The results are summarized in table 4.

TABLE 4.—*Changes in the teeth of rats on diet 411-B*[1] *with various amounts of sodium fluoride in the drinking water*

Lot no.	Number of rats	Amount of NaF in drinking water (p.p.m.)	Changes in labial surface of lower incisors
1191	4	None.	None.
1193	4	50	Very fine brown striations best seen with hand lens.
1192	4	150	Brown striations later becoming irregular.
1185	4	500	White, chalky, brittle hypoplastic enamel.

[1] 5 percent calcium carbonate in diet.

The changes in the teeth in each case were similar to the changes seen in the rats receiving the same quantity of sodium fluoride in the water without the excess calcium in the diet. It would appear, therefore, that under the conditions of the experiment the excess calcium in the diet had no appreciable effect on the gross changes produced in the teeth by sodium fluoride in the drinking water.

In view of the fact that our experiments with 500 p.p.m. of sodium fluoride in the drinking water indicated a toxic effect greater than that reported by other workers using larger amounts of sodium fluoride in the diet, a group of 4 rats was placed on a diet identical with that used in our experiments with sodium fluoride in the drinking water (diet 411) except that a solution of sodium fluoride was evaporated on the corn starch to give a final quantity of 500 p.p.m. of sodium fluoride in the diet (411-D). The animals in this lot (1189) were given distilled drinking water. The rate of growth of these animals in comparison with a similar group on diet 411-A and 500 p.p.m. of sodium fluoride in the drinking water is given in chart 1.

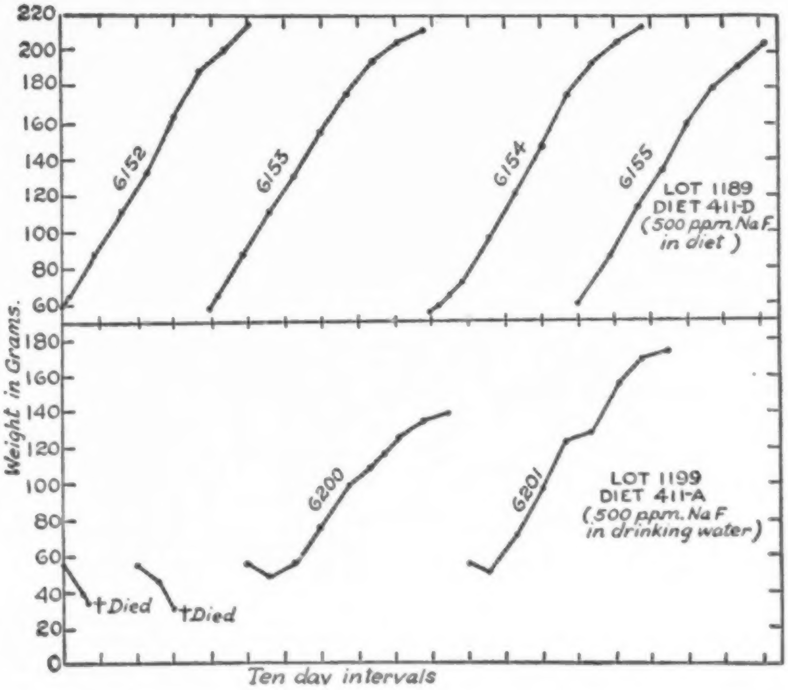
In addition, the changes in the teeth of the group on 500 p.p.m. of sodium fluoride in the diet were not as severe as those noted in the group receiving the 500 p.p.m. of sodium fluoride in the drinking water. These results therefore indicate that a given concentration of sodium fluoride in the drinking water produced a more toxic reaction than the same concentration of sodium fluoride in the diet. Whether this result is due to differences in the total quantity of sodium fluoride ingested, to more complete absorption, to a more rapid rate of absorption, or to other factors has not been definitely determined.

SUMMARY

As little as 25 p.p.m. of sodium fluoride in the drinking water of white rats produces changes in the teeth which are manifested by minute, transverse, brown striations. These striations become more pronounced as the quantity of sodium fluoride in the water is increased to 50, 75, and 100 p.p.m., respectively. When 150 p.p.m. is used, the striations lose their uniformity and become irregular brown patches. If 300 p.p.m. of sodium fluoride is used, the teeth become creamy in color, tend to fracture, and no striations are seen. If 500 p.p.m. is used, some of the animals die and the teeth of the survivors become white, chalky, and very brittle.

The addition of 5 percent of calcium carbonate to the diet failed to cause any appreciable change in the gross appearance of the teeth of rats on 50, 150, and 500 p.p.m. of sodium fluoride, respectively, in the drinking water.

CHART 1.—*Comparison of rate of growth of rats given 500 p.p.m. NaF in the diet with the rate of growth of those given the same amount of NaF in drinking water.*



Five hundred p.p.m. of sodium fluoride in the drinking water produced a relatively greater toxicity than 500 p.p.m. of sodium fluoride in the diet under the conditions of these experiments.

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Inhibition of Experimental Dental Caries By Fluoride

There is a pronounced inhibitory effect of fluoride on induced experimental dental caries in white rats. Both the occlusal fissure type and smooth surface dental caries are significantly inhibited. At a level of 50 ppm of fluoride in the drinking water, sodium fluoride, sodium fluosilicate, ammonium fluosilicate and sodium monofluorophosphate are equally cariostatic.

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OBSERVATIONS ON INDUCED DENTAL CARIES IN RATS. I. Reduction by Fluorides and Iodoacetic Acid

FRANK J. MCCLURE and FRANCIS A. ARNOLD, JR.

The communal water supply at Galesburg, Illinois, as compared with that of Quincy, Ill., was shown to have a marked beneficial effect in inhibiting human dental caries (*1*). This finding based on epidemiological evidence was tested experimentally by studying the effects of these two drinking waters on induced caries in rats. The results obtained thus far have proved inadequate to warrant definite conclusions, but the data are none the less useful to call attention to what may be an important factor in the problem of induced caries in rats, namely, a possible litter association as to caries susceptibility existing among experimental rats.

Other data in this paper, which is the first of a series of papers reporting some recent observations on rat caries made at the National Institute of Health, relate to the effect of: (*a*) 2 p.p.m. of fluorine as sodium fluoride in the drinking water; (*b*) 125 p.p.m. of fluorine as sodium fluoride in the drinking water; (*c*) 125 p.p.m. of fluorine as sodium fluoride in the ration, and (*d*) 200 p.p.m. of iodoacetic acid in the ration, together with 20 p.p.m. of iodoacetic acid in the drinking water.

Miller (*2*) reported the prevention of induced caries in rats by feeding fluorides and iodoacetic acid. His results with respect to fluorides were confirmed by Finn and Hodge (*3*). Cox, Matuschak, Dixon, Dodds and Walker (*4*) sought to show a reduced incidence of caries in rats due to fluorides contributed to the offspring by mother rats during gestation and lactation.

The first production of carious-like lesions in rats molar teeth is attributed to Shibita (*5*) who used a diet containing unground rice and greens mixed with various sugars. Coarse ground cornmeal as contained in the Hoppert, Webber and Canniff diet (*6*) will produce similar lesions. Rosebury, Karshan and Foley (*7*) have studied the etiology of this type of rat caries and have collected much data with respect to dietary variations including minerals, vitamins, fats, carbohydrates, and proteins as factors affecting the rats response to a caries-producing diet.

In the studies reported in this paper and in the succeeding papers to follow the simplified Hoppert cornmeal ration as used by Cox and his coworkers was fed, and the system of recording and scoring occlusal rat caries as proposed by Cox, Dodds, Dixon and Matuschak (*8*) was followed.

TABLE I.—*Effects of Galesburg and Quincy waters and waters containing 2 p.p.m and 125 p.p.m. fluorine as sodium fluoride*

LITTER NO.	NO. OF RATS	NO. OF RATS WITH CARIES	TEETH AFFECTED						AREAS AFFECTED						SCORE	
			Upper		Lower		Total		Upper		Lower		Total			
			No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	Total	Ave. per rat
Controls—distilled water group																
3-8-301	6	6	2	5.5	25	69.4	27	37.5	2	3.3	66	45.8	68	33.3	156	26.0
3-7-301	4	2	0	0.0	5	20.8	5	10.4	0	0.0	6	6.2	6	4.4	14	3.5
3-4-303	6	6	3	8.3	25	69.4	28	38.9	4	6.6	93	64.6	97	47.5	275	45.8
3-1-302	6	6	10	27.7	22	61.1	32	44.4	13	21.6	45	31.2	58	28.4	145	24.2
3-2-300	5	5	3	10.0	22	73.3	25	41.7	4	8.0	63	52.5	67	39.4	184	36.8
Quincy water group																
1-3-102	8	8	1	2.1	28	58.3	29	30.2	1	1.2	56	29.2	57	21.0	142	17.8
1-4-103	5	5	2	6.7	16	53.3	18	30.0	2	4.0	26	21.6	28	16.5	60	12.0
1-6-106	8	8	4	8.3	33	68.7	37	38.5	5	6.2	115	59.9	120	44.1	322	40.3
1-7-101	5	3	0	0.0	11	36.7	11	18.3	0	0.0	22	18.3	22	12.9	53	10.6
1-8-101	8	7	0	0.0	32	66.7	32	33.3	0	0.0	72	37.5	72	26.5	180	22.5
1-9-101	8	8	2	4.2	43	89.6	45	46.9	2	2.5	144	75.0	146	53.7	396	49.5
Galesburg water group																
2-12-201	5	2	0	0.0	2	6.7	2	3.3	0	0.0	2	1.6	2	1.2	3	0.6
2-11-201	4	4	1	4.2	14	58.3	15	31.3	2	5.0	30	3.1	32	23.5	71	17.7
2-10-205	6	5	1	2.8	20	55.5	21	29.2	1	1.6	51	35.4	52	25.5	139	23.2
2-9-204	5	3	0	0.0	10	33.3	10	16.7	0	0.0	25	20.8	25	14.7	65	13.0
2-2-202	9	9	17	31.5	39	72.2	56	51.9	22	24.4	138	63.9	160	52.3	432	48.0
2 ppm. fluoride water group																
4-2-401	9	9	0	0.0	25	46.3	25	23.2	0	0.0	54	25.0	54	17.6	130	14.4
4-1-401	6	5	1	2.8	20	55.6	21	29.2	1	1.6	50	34.7	51	25.0	115	19.2
4-3-401	6	6	4	11.1	27	75.0	31	43.1	7	11.7	93	64.6	100	49.0	285	47.5
125 ppm. fluoride water group																
4-5-401	7	3	0	0.0	3	3.6	3	1.8	0	0.0	4	2.4	4	1.7	6	0.9
4-6-401	9	3	0	0.0	4	3.7	4	1.9	0	0.0	4	1.9	4	1.3	7	0.8
2884-A	2	2	1	8.3	3	25.0	4	16.7	1	5.0	3	6.3	4	5.9	4	2.0
2883-A	4	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2870-A	1	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2871-A	3	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

EXPERIMENTAL

The effects of Galesburg and Quincy drinking waters and 2 p.p.m. fluorine water were tested on litters of rats whose mothers had also received these waters prior to and during gestation and lactation. The experiment included a control group given distilled water. All the breeding females were given a stock ration. The young were separated at 21–23 days of age and were fed in groups according to sex. They were given a caries-producing ration consisting of 66 percent granulated cornmeal (approximately 60 per cent of this meal was retained by a 40-mesh sieve), 30 per cent whole milk powder, 3 percent alfalfa

TABLE II.—*Summary of the effects of Galesburg and Quincy waters and waters containing 2 p.p.m. and 125 p.p.m. fluorine as sodium fluoride*

TREATMENT		NO. OF RATS	NO. OF LITTERS	RATS WITH CARIES	CARIOUS TEETH Upper	CARIOUS TEETH Lower	CARIOUS TEETH Total	AREAS AFFECTED Upper	AREAS AFFECTED Lower	AREAS AFFECTED Total	SCORE* TOTAL
Control (distilled water)	No.	27	5	25	18	99	117	23	273	296	774
	Per cent	—	—	92.6	11.1	61.1	36.1	8.5	42.1	32.2	—
	Ave. rat**	—	—	—	0.7	3.7	4.3	0.9	10.1	11.0	31.0
Quincy water	No.	42	6	39	9	163	172	10	435	445	1153
	Per cent	—	—	92.9	3.6	64.7	34.1	2.4	43.2	31.2	—
	Ave. rat	—	—	—	0.2	3.9	4.1	0.2	10.4	10.6	29.6
Galesburg water	No.	29	5	23	19	85	104	25	246	271	710
	Per cent	—	—	79.3	10.9	48.9	29.9	8.6	35.3	27.5	—
	Ave. rat	—	—	—	0.7	2.9	3.6	0.9	8.5	9.3	30.9
2 ppm. F in water	No.	21	3	20	5	72	77	8	197	205	530
	Per cent	—	—	95.2	4.0	57.1	30.6	3.8	39.1	28.7	—
	Ave. rat	—	—	—	0.2	3.4	3.7	0.4	9.4	9.8	26.5
125 ppm. F in water	No.	26	6	8	1	10	11	1	11	12	17
	Per cent	—	—	30.8	0.6	6.4	3.5	0.4	1.8	1.4	—
	Ave. rat	—	—	—	0.04	0.4	0.4	0.04	0.4	0.4	2.1

* Score averages are based on the number of rats having caries.

** Averages for carious teeth and areas affected are based on the total number of rats in the group.

meal (all of which passed a 60-mesh sieve utilized to remove particles of sand which may contaminate alfalfa meal) and 1 percent sodium chloride. The drinking waters of these 4 groups were the same as received by their mothers. Another group was given 125 p.p.m. fluorine in the drinking water but no added fluorine was given to the parents of these rats. Initial and final weights were determined for each rat. At the end of 15 weeks the rats were killed, the heads and leg bones cleaned of all adhering tissue and dried. The molar teeth were examined under a low power microscope (magnification × 22.5), the visible cavities being recorded and scored on a chart, described and illustrated in the article by Cox, Dodds, Dixon and Matuschak (*8*). Only the cavities as seen in unsectioned teeth were recorded. The examination and scoring of each set of teeth was checked by both authors.

The experimental plan to test the effects of 125 p.p.m. of fluorine in the ration, and iodoacetic acid in the ration and drinking water, did not involve any prior treatment of the breeding stock. Young stock rats were taken at 21 days of age and litter mates were divided among three groups, i.e., control group, one fed fluorides, and the other given iodoacetic acid. Each litter of rats was represented in each group by one or two litter mates. No distinction was made as to sex, which does not appear to be a factor influencing rat caries.

The rations for this experiment consisted of the basal ration described above with certain additions. Fluoride (125 p.p.m. as NaF) was added to the ration in the form of a dilute solution sprinkled over the cornmeal and thoroughly mixed with it. This cornmeal was dried in a current of warm air to its original weight. Iodoacetic acid in solution was added to cornmeal in a similar manner so that the final ration would contain 200 p.p.m. iodoacetic acid. The cornmeal for the control ration was treated with a quantity of distilled water equal to that used for a test ration, and it was similarly dried to its original weight.

The control group and the fluoride group received distilled water for drinking. The iodoacetic group received drinking water containing 20 p.p.m. of iodoacetic acid. These levels of fluoride and iodoacetic acid are similar to the quantities fed by Miller (*2*). It was not considered desirable to add yeast to the diet of rats receiving iodoacetic acid, as was done by Miller (*2*) to obviate a possible toxic action of iodoacetic (*9*). Judging from our growth rates and from gross observations based on all the rats in the control and test groups, this level of iodoacetic acid as fed was innocuous. The rats were kept on the above rations and drinking waters for 15 weeks, when they were killed and the teeth examined as above.

DATA

The amount and degree of caries in the rats may be judged from the data appearing in the accompanying tables, tables I, II, and III. As may be seen, the bases for comparison of the various groups of rats are: (*a*) the number and

TABLE III.—*Effects of sodium fluoride in ration, and iodoacetic acid in ration and drinking water*

TREATMENT		NO. OF RATS	NO. OF LITTERS	RATS WITH CARIES	CARIOUS TEETH Upper	CARIOUS TEETH Lower	CARIOUS TEETH Total	AREAS AFFECTED Upper	AREAS AFFECTED Lower	AREAS AFFECTED Total	SCORE* TOTAL
Control	No.	25	14	19	7	67	74	9	125	134	264
	Per cent	—	—	76.0	4.66	44.67	24.66	3.60	20.83	15.76	—
	Ave. rat**	—	—	—	0.28	2.68	2.96	0.36	5.0	5.36	13.89
125 ppm. F as sodium fluoride in ration	No.	25	14	3	1	2	3	1	2	3	4
	Per cent	—	—	12.0	0.66	1.32	1.0	0.40	0.33	0.35	—
	Ave. rat	—	—	—	0.04	0.08	0.12	0.04	0.08	0.12	1.33
Iodoacetic acid (200 ppm. in ration and 20 ppm. in water)	No.	24	12	8	2	17	19	2	20	22	36
	Per cent	—	—	33.3	1.39	11.80	6.60	0.83	3.47	2.70	—
	Ave. rat	—	—	—	0.08	0.71	0.79	0.08	0.83	0.91	4.50

* Score averages are based on the number of rats having caries.

** Averages for carious teeth and areas affected are based on the total number of rats n each group.

percent of rats in each group with caries, i.e., having one or more carious teeth; (*b*) the number and per cent of carious teeth (molars) per group; (*c*) the number and percent of affected tooth areas per group as indicated by Cox's chart, and (*d*) the average total score of the rats having caries based on Cox's chart which permits an evaluation of the size of each cavity developed in any of the specified areas by giving it a numerical score of 1, 2, or 3. Average rat scores based on a litter as a group are also shown in table I.

RESULTS OF EXPERIMENT

The results which pertain to the Galesburg water, Quincy water, and the 2 p.p.m. fluoride water groups, and their distilled-water control group, are the first extensive published data illustrating what appears to be a litter membership factor involved in the production of rat caries [diagnosed more specifically as occlusal caries by Cox, Dodds, Dixon and Matuschak (*8*)]. For example a somewhat different caries picture is apparent among the nine rats of litter 2-2-202, table I, each rat showing four to seven affected teeth, as compared with the five litter mates of litter 2-12-201, table I—only two rats of this later litter were carious having but one carious tooth each. It thus appears from our limited data that certain variations in rats grouped as to litter membership are truly abnormal as compared with the distribution of the data selected at random. Such results are indicative of a significant finding and the entire question deserves further examination and study. There is no information that a specific caries response is a hereditary characteristic of all the litters of a given male and female mating, or is due to variable conditions which may occur during gestation and lactation or during the actual caries-producing period.

The summary data as shown in table II relates to all the rats of a group, disregarding the rats litter membership. This does not appear to be the logical treatment of such data which possesses a natural grouping in accordance with prenatal and lactation effects as well as by certain observed litter membership tendencies toward caries. The coefficient of variation for the data where the result per litter is a single item is quite large, and many more litters per group than shown here would be required to demonstrate significant differences on this basis. Thus using the litter average data for rats with or without caries, based on a total of 19 litters (all litters except those given 125 p.p.m. fluorine), there is obtained a coefficient of variation of 22.5. Referring to the method proposed by Mitchell and Grindley (University of Illinois, Agricultural Experiment Station, Bulletin No. 165, July 1913. The Element of Uncertainty in the Interpretation of Feeding Experiments by H. H. Mitchell and H. S. Grindley) for calculating the number of animals that should be included in experimental lots on the basis of the variability of the individual data, it may be shown that roughly 35 litters per group would be required to demonstrate a 10 percent significant difference between groups made up of litter units. A discrepancy may reside in other data of this type based on group averages, particularly where the number of rats in a group is small and no data are given as to litter distribution (*4, 7*). In all due fairness to such group data it must be noted, however, that up until the time the pronounced effects of fluorides and iodoacetic acid on caries were reported (*2, 3*), group averages generally did not show very significant differences among groups receiving different treatments. This may be an indication that such group averages were obtained with proper recognition of litter membership, but it may also indicate a need for other comparisons being made with more animals, particularly where small differences are presented as representing significant results.

The results relative to the effects of Galesburg water, Quincy water, and 2 p.p.m. fluoride water do not show any differences which are statistically significant. The data relative to 125 p.p.m. of fluorine justify the conclusion that this level of fluorine in the drinking water has significantly inhibited caries development in our experimental rats.

The response of rats given a caries-producing diet to the beneficial effects of 125 p.p.m. of fluorine in the ration, and to the effects of iodoacetic acid in the ration and drinking water, is strikingly illustrated by the data presented in table III. The reduction of caries in the fluoride group below that established by their litter mates in the control group is unquestionable, as brought out by all items tabulated to establish the caries picture for the groups. In these groups it is to be noted that each rat in a test group is represented by a litter mate in the control group, and 12–14 litter trios are involved in the study. Differences between the control group and each of the test groups are both more than 100 percent. There can be no doubt that this is a significant finding. A significant result seems now established as an effect of iodoacetic acid. Both these results are in confirmation of previous results first reported by Miller (*2*). In comparing this experiment with Miller's report, attention might be called to the fact that yeast was introduced into his iodoacetic acid ration.

It is interesting to note the difference in the rats of the two control groups shown in tables II and III. The control rats represented in table III, as compared with table II, were from different breeding strains which may account partly for the different control results. It is to be noted also that the cornmeal for the control rats in table III was treated with distilled water and then dried, which may have modified its caries-producing properties. It is to be remembered also that an uncontrolled difference in fluorine intake from the basal rations fed these two control groups could account for some difference in response.

DISCUSSION

In the present state of our knowledge, there are few adequate available data directly suited to explain these observed effects of fluoride and iodoacetic acid on induced rat caries. Laboratory observations such as these have followed the epidemiological studies regarding the association of certain communal water supplies known to contain fluorides, and human dental caries (*10*). A role of fluorine contained in the drinking water is definitely implied in these studies. This current new approach to the study of caries not only has furnished important facts regarding dental caries, but it gives rise to the possibility of partial control of human dental caries.

These observed anticaries effects of fluorides have at least two possible modes of action, either one of which still lacks adequate experimental confirmation. The activity of fluorine as an antienzymatic agent lends weight to the belief that fluorine may prevent bacterial degradation of carbohydrates in food deposits. Fluorine, when deposited in the enamel particularly, as shown by Armstrong and Brekhus (*11*), may, on the other hand, bring about a more acid-resistant, possibly antienzymatic tooth material. The effect of a modified saliva and the involvement of more subtle surface phenomena can not be dismissed as possible factors also.

There are certain little understood facts which relate to the production of caries in experimental rats. In the minds of all workers in this field it is unfortunate first, that a coarse, hard particle diet must be used to produce rat caries because it introduces the uncertain element of fracture; secondly, a diet containing a natural ingredient such as whole ground corn leaves something to be desired by way of adequate dietary control. On the other hand, it is not improbable that the efficacy of the coarse particles of cornmeal or rice may as well be referred to as a food impaction phenomena. In fact the prevention of rat caries by fluorine in itself may be taken as evidence against the belief that a mechanical factor is the essential feature of these caries-producing diets. These facts, by assumption at least, relate the etiology of rat caries to that of human dental caries.

SUMMARY

A considerable amount of data relative to the effects of Galesburg (Ill.) drinking water, Quincy (Ill.) drinking water, and water containing 2 p.p.m. fluorine as sodium fluoride on induced cornmeal rat caries has been accumulated. These data have been found inadequate to demonstrate different effects, but they call attention to a litter membership factor in rats with respect to their caries susceptibility.

The presence of 125 p.p.m. of fluorine as sodium fluoride in the food or water and the presence of 200 p.p.m. of iodoacetic acid in the food, together with 20 p.p.m. of iodoacetic acid in the drinking water, significantly reduce the production of caries in rats fed a caries-producing corn meal diet.

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OBSERVATIONS ON INDUCED DENTAL CARIES IN RATS. II. The Effect of Subcutaneous Injection of Fluoride

FRANCIS A. ARNOLD, JR. and FRANK J. MCCLURE

Epidemiological evidence (1), (2), (3), and experimental studies in rats (4), (5), (6), have shown that the process or processes involved in the production of dental caries can be altered by the presence of fluorine in the drinking water or in the food. It has been suggested that the fluoride ion acts either as an antienzymatic agent or produces a more resistant tooth substance; whether this action is local, systemic, or both, is not known.

Dean, Jay, Arnold, McClure and Elvove (2) have pointed out that the epidemiological evidence cannot be adequately explained on the presence or absence of dental fluorosis; however, at Bauxite, Ark., mottled teeth resisted dental caries attack even though the population had been using a fluoride-free drinking water for the past 12 years (7). Cheyne has interpreted his results (8) to show that the action of fluorine on dental caries in rats was not dependent on the presence of saliva. McClure (9) has shown that the caries resistance of rats is correlated with the amount of fluorine in the diet; also the fluorine content of the molar teeth of these rats

TABLE I.—*Summary of results showing the effect of injected fluoride and water containing 10 p.p.m. F as NaF on induced caries in rats*

TREATMENT	NO. OF RATS	NO. OF LITTERS	RATS WITH CARIES	CARIOUS TEETH			AREAS AFFECTED			TOTAL SCORE	SCORE PER CARIOUS RAT
				Upper	Lower	Total	Upper	Lower	Total		
Controls (distilled water)											
No.	23	21	23	7	70	77	7	145	152	361	—
Per cent	—	—	100	5.1	50.7	27.9	0.03	26.3	19.4	—	—
Ave. rat	—	—	—	0.3	3.0	3.3	0.3	6.3	6.6	15.7	15.7
Injection (dist. water and inject. of fluoride)											
No.	24	21	20	11	53	64	15	138	153	388	—
Per cent	—	—	83	7.6	36.8	22.2	0.06	24.0	18.8	—	—
Ave. rat	—	—	—	0.5	2.2	2.7	0.6	5.8	6.4	16.2	19.4
Fluoride water (10 ppm. F in water)											
No.	20	19	16	3	50	53	3	110	113	279	—
Per cent	—	—	80	2.5	41.7	22.1	0.02	22.9	16.6	—	—
Ave. rat	—	—	—	0.2	2.5	2.7	0.2	5.5	5.7	13.9	17.4

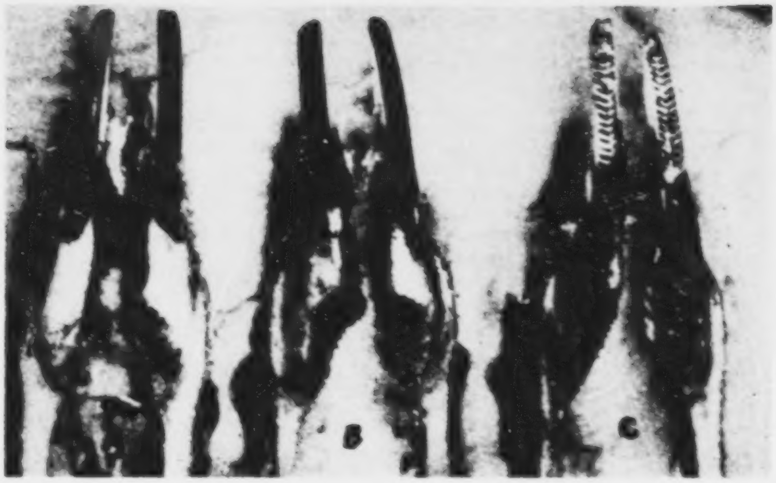

FIGURE 1.—*Lower incisor teeth of rats; A.—control, B.—10 p.pm. F. as NaF., C.—Injection of 0.5 mg. F. as NaF.*

changes in direct proportion to the amount of fluorine in the diet. None of this evidence permits conclusions to be made as to whether the action of fluorine on dental caries is local, systemic, or associated with the tooth structure.

The present experiment was planned to eliminate the local action of the fluorine so far as it is related to the presence of this element in the food or drinking water. To this end, the fluorine was administered by subcutaneous injections of NaF, and the dental caries experience of the injected rats was compared with that of a control group and a group reciving 10 p.p.m. F in drinking water.

EXPERIMENTAL METHOD

The rats used in this experiment were separated from their mothers at 21–23 days of age and kept on a non-caries producing stock ration and distilled water until they had reached more than 30 days of age. The average age of each group when placed on the experiment was: control—32.8 days, 10 p.p.m. group—32.7 days, injection group—41.3 days. After this period litter mates were distributed among three groups, i.e., control, injection, and fluoride water. All rats were given food and water ad libitum. The food used for all rats was a caries-producing cornmeal ration consisting of 66 percent granulated cornmeal (approximately 60 percent of this meal was retained by a 40 mesh sieve), 30 percent whole milk powder, 3 percent alfalfa meal (all of which passed a 60 mesh sieve) and 1 percent sodium chloride. The control group, and the injection group received distilled water; the third group received drinking water which contained 10 p.p.m. F as NaF. The injection group received subcutaneous injections of 1 cc. of a solution of NaF equal to 0.5 mg. F every second day for the first 24 days and daily thereafter, making a total of 86 injections for each rat.

The molar teeth were examined under a low power microscope (magnification × 22.5), the visible cavities being recorded and scored on a chart similar to the method described and illustrated by Cox, Dodds, Dixon and Matuschak (*10*). Only the cavities as seen in unsectioned teeth were recorded, grouping male and female results, because sex differences did not warrant separate classification. These results are shown in table I.

The extent of absorption of fluorine may be judged by observing the fluorine content of the incisor teeth and by noting the degree of dental fluorosis present. Photographs of the incisor teeth, representative of each group, are shown in figure 1.

The crowns of the molar and incisor teeth of the upper and lower right jaws were removed and pulverized to pass through a 60-mesh sieve. The enamel and dentin were separated, using the method described by Manly and Hodge (*11*). The fluorine content of these enamel and dentin samples was determined by distillation from perchloric acid and titration with thorium nitrate. Details of the procedure followed may be found in an article by McClure (*12*). The results of these determinations are shown in table II.

DISCUSSION

The results as presented in tables I and II seem to indicate two major findings, both of which require further discussion.

First: Fluorine, when injected subcutaneously in these rats, did not increase their resistance to induced dental caries. According to the data as obtained, the injected group of rats had as much dental caries as either the control or the 10 p.p.m. fluoride water group. This negative effect of the injected fluoride was accompanied by an average daily intake of approximately 3 mg. of injected fluorine per kilogram of body weight. This amount approximates the average daily fluorine intake of rats whose drinking water contains 40–50 p.p.m. F. Judging from the quantity of fluorine retained by the incisor teeth as indicated by the degree of dental fluorosis and by quantitative analyses, a greater percent of fluorine was absorbed from injection of sodium fluoride than from similar quantities of sodium fluoride given in food or water. According to the results obtained by McClure (*9*), drinking water containing 40–50 p.p.m F should produce a signifi-

TABLE II.—*Results of determinations of the ash and fluorine content of the crowns of rat incisor and molar teeth*

Treatment	No. of rats	Incisors				Molars			
		Enamel		Dentine		Enamel		Dentine	
		% Ash	% F	% Ash	% F	% Ash	% F	% Ash	% F
Controls (distilled water)	23	95.69	.0036	79.45	.0046	95.18	.0034	79.03	.0089
Fluoride water, 10 p.p.m. in water	20	95.01	.0159	79.01	.0494	95.41	.0128	79.38	.0414
Injection distilled water plus injection of fluoride	24	95.49	.0418	79.44	.1301	95.20	.0131	79.13	.0677

cant reduction in the amount of induced dental caries, however, the results on the injected rats indicate no such reduction. Attention is called to the fact that the group of rats receiving water containing 10 p.p.m. F show little, if any, significant reduction in dental caries. Whether this is due to the age of the rats, or whether 10 p.p.m. F represents a borderline amount with respect to induced rat caries as is shown by McClure's results is not known (*9*).

The above result with respect to injected fluoride indicates that the action of fluorine in reducing dental caries in rats is possibly more closely related to local or oral factors than to some systemic influence. This finding makes it appear improbable that a significant reduction of induced rat caries by fluorine comes through the saliva; a finding which is in accordance with the results reported by Cheyne (*8*) for rats with extirpated salivary glands.

Second: The results as shown in table II indicate that fluorine, when injected subcutaneously in rats, increased the amount of fluorine in the enamel and dentin of both the incisor and molar teeth. This increase is related to the fluorine intake of the rat since the teeth of the fluoride water group also show a fluorine increase but the increase was not as great as was found in the teeth of the injected rats. It may also be noted that the quantity of fluorine absorbed in the dentin of both the incisor and molar teeth was greater than in the enamel. These results based on fluorine analyses permit the assumption that the composition of the enamel and dentin of molar teeth of rats may be altered by way of the blood stream through internal surface phenomena even after the crowns of these teeth have erupted in place. According to Addison and Appleton (*18*) and Mellanby (*19*) all the molars would be erupted at approximately 35 days.

The above statement must be qualified because of the possibility that some, if not all, of the extra fluorine deposited in the enamel may have come from the saliva. Considering the low results obtained by McClure (*13*) as to the amount of fluorine in human saliva, and the fact that according to Wills (*14*) the time during which radio-active fluorine was present in the saliva of injected cats was very short, it appears unlikely that the observed increases of fluorine in the enamel of the injected rats were due to an adsorption of this element from the saliva. These observed results pertaining to the injected fluoride and its effect on enamel fluorine make it seem doubtful that an increase in the fluorine content of the enamel of rats given a fluoride drinking water comes entirely through the surface as Perry and Armstrong (*15*) have recorded.

According to the data presented in tables I and II it appears that even though the fluorine content of the molar teeth of rats was increased, there was no proportionate increase in the resistance of these teeth to dental caries. This finding may not be in contradiction to Cox, Matuschak, Dixon, Dodds and Walker (*16*) and Armstrong and Brekhus (*17*) whose results suggest that optimum amounts of fluorine in the teeth make for increased resistance to caries. In other words, fluorine in this case has been added following eruption and its action against caries may not be similar to the action of fluorine which has been added during the formative period of the tooth.

In general, the results for injected fluorides thus far obtained warrant the assumption that the action of fluorine on induced dental caries in rats is associated with local factors, principally those factors associated with the diet of the rat.

SUMMARY

Subcutaneous injection of sodium fluoride produced no significant reduction in induced dental caries of rats.

The fluorine content of the enamel and dentin of the incisor and erupted molar teeth of rats was increased both by subcutaneous injection of sodium fluoride and by feeding water containing 10 p.p.m. F.

The observations on the injected rats would seem to rule out any systemic influence of fluorine on induced rat caries when the agent (F) is introduced during the post eruptive tooth period.

The results of this experiment indicate that although the fluorine content of the molar teeth of rats injected with sodium fluoride was increased, these teeth did not show increased resistance to caries.

Acknowledgment is made to Dental Surgeon H. T. Dean for advice and suggestions used in this experiment.

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OBSERVATIONS ON INDUCED CARIES IN RATS. III. Effect of Fluoride on Rat Caries and on Composition of Rats' Teeth

F. J. McClure

Epidemiological studies relate a partial prevention of human dental caries to the occurrence of fluorides in drinking water (Dean, 1938; Dean, Jay, Arnold, McClure and Elvove, 1939; Dean, Jay, Arnold and Elvove, 1941a). Induced dental caries in rats also is inhibited by the ingestion of fluoride (Miller, 1938; Finn and Hodge, 1938; McClure and Arnold, 1941). These effects may be due to: (*a*) inhibition of bacterial action on food residues (Lundsgaard, 1930), or dental tissue (Robison and Rosenheim, 1934); (*b*) a systemic effect of fluoride on tooth formation (Armstrong and Brekhus, 1938; Cox, Matuschak, Dixon, Dodds and Walker, 1939; Volker, 1939; Dean, Jay, Arnold and Elvove, 1941b), or (*c*) a modified saliva (Cheyne, 1940; Wills, 1940; McClure, 1941).

Enamel may become hypoplastic (mottled) as a result of fluoride ingestion during the period of dental calcification. Fluoride has no known post-eruptive effect on human dental enamel, although certain data (Perry and Armstrong, 1941) indicate that in rats the oral enamel surface may adsorb fluorides from drinking water. It remains to be demonstrated that post-eruptive ingestion, or a local oral treatment with fluoride, will add significant quantities of fluorine to human enamel, or by some other action inhibit human dental caries.

EXPERIMENTAL

The experimental plan follows a previous study (McClure and Arnold, 1941). Groups of rats aged 23-25 days, 16 to 23 litter mates in control and corresponding test groups, were on a test period of 15 weeks. A cornmeal caries-producing diet was fed to all groups, ad libitum. The caries effect of this diet is generally attributed to the size of the corn particle (Lilly and Wiley, 1934). The diets as used averaged 0.3-0.5 p.p.m. fluorine. Except in two test groups fluoride was added via drinking water. Water intake was not controlled, the results being based, therefore, on fluoride concentration of water or food, and on fluoride given by subcutaneous injection. Comparisons are made between a test group and its corresponding control (McClure and Arnold, 1941).

After examining the molar teeth for occlusal caries (Cox, Dodds, Dixon and Matuschak, 1939), pooled samples of the crowns of the molars and the erupted part of the incisors of the various groups were pulverized to pass a 60-mesh sieve, the dentine and enamel separated (Manley and Hodge, 1939), and fluorine (McClure, 1939a) and ash determined. Pooled samples of the combined dentine and enamel of carious and noncarious rats' molars were analyzed for ash, calcium, phosphorus, and fluorine. Certain of the data was obtained from other experiments (McClure and Arnold, 1941; Arnold and McClure, 1941).

RESULTS

Fluorides equaling 50 and 100 p.p.m. fluorine in water, and 125 p.p.m. in food, inhibit rats caries decisively (table 1 and

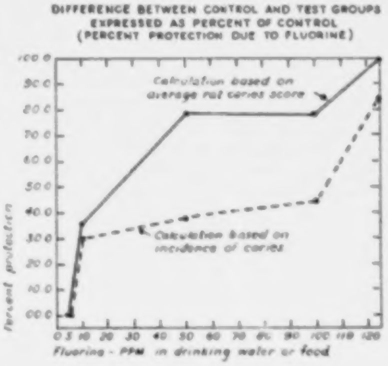

FIGURE 1

TABLE 1.—*Effect of fluoride on rat caries*

TREATMENT	NUMBER OF RATS PER GROUP	RATS WITH CARIES	CARIOUS MOLAR TEETH	CARIOUS MOLAR TOOTH AREAS	AVERAGE CARIES SCORE[1]	
					All rats	Carious rats
		per cent	*per cent*	*per cent*		
Control A	23	95.6	27.5	20.6	17.1	17.9
10 ppm. F. in water	23	69.6	14.9	10.5	8.2	11.8
Control B	27	100.0	35.2	31.4	27.6	27.6
10 ppm. F. in water	24	79.2	26.7	18.8	15.6	19.7
100 ppm. F. in water	26	57.7	13.8	7.8	6.2	16.1
Control C	26	80.8	24.4	20.6	18.0	22.3
5 ppm. F. in water	25	88.0	24.3	20.0	17.9	20.3
50 ppm. F. in water	25	52.0	10.7	5.5	4.0	7.6
Control D	25	76.0	24.7	15.8	10.6	13.9
125 ppm. F. in food	25	12.0	1.0	0.4	0.2	1.3
Control E	23	100.0	27.9	19.4	15.7	15.7
10 ppm. F. in water	20	80.0	22.1	16.6	14.0	17.4
NaF injected	24	83.0	22.2	18.8	16.2	19.4

[1] There are a total of thirty-four areas distributed over the twelve molar teeth in which areas cavities are most likely to occur. Depending on its size, each cavity is given a score of 1, 2, or 3 (see Cox, Dodds, Dixon and Matuschak, '39).

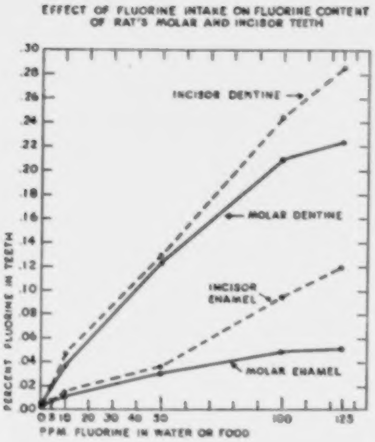

FIGURE 2

fig. 1). It may be concluded that 10 p.p.m. fluorine in water also inhibited caries as studied. Thus, in 56 possible comparisons between litter mate control and test rats, the 10 p.p.m. fluorine rat showed a lower caries score in 38 instances. The odds are 1 to 259 against this being a fortuitous happening (Student, 1925). Rats ingesting 5 p.p.m. fluorine in water were not different from their controls. The effect of injected fluorine on induced rat carries appears to be negative, although these rats received an average daily intake of fluorine approximately equal to 50 p.p.m. of fluorine in drinking water.[2]

The ash of molar and incisor dentine and enamel was not affected by fluoride. Similarly there was no difference in ash, calcium, phosphorus, and fluorine of mixed dentine and enamel of molars of carious rats, as compared with non-carious rats (table 2).[3]

TABLE 2.—*Ash, calcium, phosphorus, and fluorine in molar teeth of carious and non-carious rats*

TREATMENT	NUMBER OF RATS	AVERAGE CARIES SCORE	COMPOSITION OF MIXED DENTINE AND ENAMEL			
			Ash	Ca	P	F
			per cent	*per cent*	*per cent*	*per cent*
Control A non-carious	5	0.0	78.2	29.1	15.1	.0031
Control A carious	17	17.9	77.0	28.9	14.7	.0035
10 ppm. F. non-carious	10	0.0	77.7	28.9	14.8	.0297
10 ppm. F. carious	13	11.8	75.3	27.7	14.1	.0291
Control B carious	27	27.6	76.9	28.9	14.4	.0032
10 ppm. F. non-carious	6	0.0	78.3	28.7	14.7	.0339
10 ppm. F. carious	18	19.7	77.5	28.5	14.9	.0365
100 ppm. F. non-carious	12	0.0	77.4	29.4	14.5	.1866
100 ppm. F. carious	14	16.1	77.4	29.1	14.8	.2009
Control C non-carious	5	0.0	79.1	28.8	15.4	.0046
Control C carious	21	22.3	77.5	28.5	14.8	.0035
5 ppm. F. non-carious	4	0.0	79.8	29.4	15.2	.0192
5 ppm. F. carious	21	20.3	76.8	28.6	14.5	.0245
50 ppm. F. non-carious	13	0.0	77.4	29.1	14.9	.1140
50 ppm. F. carious	12	7.6	78.3	29.1	14.9	.1069
Control D non-carious	8	0.0	79.2	29.8	14.9	.0025
Control D carious	17	13.9	78.3	29.7	14.6	.0030
125 ppm. F.[1] non-carious	22	0.0	78.9	29.7	15.0	.1842

[1] Fluoride in food.

DISCUSSION

The course of prevention by fluoride of induced rat caries (fig. 1) appears to support an antienzymatic mode of action. Inhibition occurred at the low level of 10 p.p.m. of fluorine; it was equally pronounced at 50 p.p.m. and 100 p.p.m., and was practically complete at 125 p.p.m. Since the observed action was not similarly proportional to fluoride deposited in the teeth, there is the suggestion of an effect on reactions occurring locally within the oral cavity. In this connection also, it may be noted that comparison of the two curves (fig. 1) indicates fluoride was perhaps less effective in preventing the initiation of caries (caries incidence) than in retarding its development (caries score).

On the basis of indirect evidence, the etiology of rat caries itself appears to involve an enzyme action within the oral cavity. Thus iodoacetic acid, which, like fluoride, is an active antienzymatic substance, inhibits rat caries (Miller, 1938; McClure and Arnold, 1941). Likewise fluoride which does not reach the oral cavity, i.e., when given by injection, is not caries-inhibitory (Arnold and McClure, 1941). In conjunction with the indicated antienzymatic effects of fluoride, a bactericidal effect on oral flora is not eliminated.

Results of fluorine analysis of the rats' teeth demonstrate a considerable retention of fluorine by molar dentine and enamel as occurring after tooth eruption (fig. 2). In regard to the rat's incisors, which are continuously erupting, deposition of fluorine is not unusual (McClure, 1939b). However, the similarity of the added quantities of incisor fluoride and molar fluoride particularly for 5, 10, and 50 p.p.m. rats, implies the acquisition of fluorine by incisors following the developmental stage.

The rat's first and second molar teeth are fully erupted by the 25th day; the third molars are fully formed at this time, but are not erupted until about the 30th day. The apposition of primary dentine in rats' molars (Hoffman and Schour, 1940) may possibly account for a part of the fluorine increment in molar dentine. Molar enamel growth, however, is presumably complete at about 10 days of age (Hoffman and Schour, 1940). A posteruptive enlargement of rat molars as reported by Donaldson (1934) was not confirmed by Wood (1935) nor by Hoffman and Schour (1940).

It is particularly significant in these regards that the molar enamel of fluoride injected rats averaged 0.0131 percent fluorine as compared with 0.0034 percent fluorine for controls (Arnold and McClure, 1941). Since it is doubtful that this increase in enamel fluoride was due to adsorption from saliva (McClure, 1941; Willis, 1940), these data seemingly indicate that the erupted dentine and enamel retained fluoride from the systemic circulation. Other evidence in the data supports this belief. Thus, the ratio of fluorine in dentine and enamel for molars and incisors is as follows:

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[2] These injection rats (Arnold and McClure, '41) received 0.5 mg. fluorine as sodium fluoride injected subcutaneously every other day for the first 24 days and every day thereafter for the remaining experimental 75–76 days. At the start of the regular injection period, these rats averaged 41.3 days old, as compared with their controls aged 32.8 days, a delay caused by a local irritation which developed at the site of the first injection. An age factor therefore might have influenced these older rats slightly in caries response. This group of rats, however, did not differ significantly from the controls.

[3] Samples of mixed dentine and enamel, as prepared from carious teeth, may have contained a less than normal quantity of enamel as a result of their carious condition. This would tend to decrease the ash results abnormally on these samples.

Fluoride treatment (p.p.m.)	10	10	10	50	100	125
Molar teeth (ratio)..........	3.74	3.23	3.23	3.96	4.20	4.31
Incisor teeth (ratio).........	2.52	3.43	3.11	3.47	2.59	2.58

This relatively constant proportion of fluorine desposited in these two dental tissues should doubtless be ascribed to a regular systemic retention, which is seemingly unaffected by oral surface adsorption of fluoride.

The property of the oral enamel surface as an adsorbent of water-borne fluoride has been tested recently by Perry and Armstrong (1941). Their data give some evidence that enamel has this property. Contrary to the findings of this report, however, Perry and Armstrong (1941) found no increase in fluorine in erupted rat molar dentine. Perhaps the age of their rats (mature male rats averaging 250 gm.) and the duration of their experiment (60 days) may account for the different results obtained.

Posteruptive deposits of fluorine may not increase the tooth's caries resistance. Thus, although fluorine increased in the molars of injected rats, caries remained the same as in control rats. A similar result applies to rats receiving 5 p.p.m. of fluorine in water. Also the fluorine in the molars of three groups of rats given 10 p.p.m. fluorine water is not proportional to the observed caries prevention. At the present time, therefore, it seems unlikely that these secondary posteruptive deposits of fluorine exerted a significant effect on the development of rat caries. Except for the injection group, perhaps any effect of tooth fluoride may have been masked by food or water-borne fluoride.

SUMMARY

A minimum of 10 p.p.m. of fluorine in drinking water gave partial protection against occlusal rat caries. Approximately 80 per cent protection resulted from both 50 p.p.m. and 100 p.p.m. fluorine in water. It is suggested that fluorine acts to inhibit rat caries by antienzymatic local action within the oral cavity.

The pooled molar teeth of groups of carious rats were not different from those of noncarious rats in content of ash, calcium, and phosphorus, and in fluorine which was deposited posteruptively.

The post-eruptive addition of fluorine to rats' molar teeth occurred in relatively large quantities and is regarded as occurring independent of oral enamel surface adsorption of fluoride. A significant effect of fluoride acquired after tooth eruption on induced rat caries was not evident.

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OBSERVATIONS ON INDUCED CARIES IN RATS. IV. Inhibiting Effect of Fluoride Ingested Posteruptively and Prior to the Caries Producing Diet

FRANK J. MCCLURE

The posteruptive increase of fluorine in dentin and enamel has been observed in rats (*1, 2*) and in an adult dog (*3*). Fluorine has been found deposited posteruptively by the rat in molar enamel but not in dentin (*4*). *In vitro* experiments show powdered enamel and dentin (*5, 6*), as well as bone tissue (7) to have the property, in common with pure mineral phosphates (*8, 9, 10*), of adsorbing fluoride.

The above *in vivo* experiments (*1, 2, 3*) are interesting from several aspects. First: They indicate that dentin and enamel may be modified chemically during posteruptive tooth life or after calcification has been completed. Second: Although fluorine was ingested in quantities varying from 10 p.p.m. to 125 p.p.m. by rats (*1*) a relatively constant proportion between fluorine retained by the enamel and fluorine retained by the dentin was maintained, i.e., about 3.5 to 4.0 times as much fluorine was found present in the dentin as in the enamel. This would seem to be due to an orderly systemic process. It advances the hypothesis that similar chemico-physical properties may be attached to both adult enamel and to adult dentin. Third: The retention of ingested fluoride by teeth posteruptively raises the question of the relation of such secondary fluorine deposits to caries resistance.

Epidemiological evidence has demonstrated the caries inhibitory properties of fluoride in water ingested during the period of tooth calcification (*11, 12, 13, 14*). An important point to emphasize in these studies (*13, 14*) is the distinction made between certain minimum quantities of fluorine which seemingly improve the dental tissue, and somewhat higher levels, which produce the hypocalcification or hypocalcification and hypoplasia known as endemic dental fluorosis (mottled enamel). On the basis of physiological effects, it also seems necessary to differentiate between at least two forms of dental tissue fluoride, i.e., fluorine acquired during the formative life of the tooth (primary fluoride) and fluorine acquired during adult or posteruptive tooth life (secondary fluoride). There is the possibility of a third form of fluoride, i.e., fluorine acquired by the exposed oral enamel through surface adsorption (adsorbed secondary enamel fluoride) (*4*).

The adaptation of some form of fluoride therapy to combat dental caries in adults is a much discussed question. A fluoride prophylaxis, using either a fluoride bearing mouth wash or dentifrice, or more particularly by means of a topical application of fluoride, seemingly relies for its success first, on the incorporation of secondary fluorine in the exposed enamel in the oral cavity, and second, on the possibility that caries resistant properties may be engendered in the tooth by adsorbed secondary enamel fluoride. These are largely unexplored hypotheses. As already indicated, the retention of fluorine by the exposed enamel in the oral cavity has some promising aspects, but it is as yet an uncertain and unproven phenomenon. Two clinical trials with children (*15, 16*) and one experimental rat study (*17*) have attempted to test the actual effect of a topical treatment with fluoride. In these studies there were indications of some benefits. More extensive data including different age groups are needed to prove the value of this therapy.

Certain experimental studies pertaining to induced rat caries, as reported herein, seem to indicate that caries inhibitory properties are acquired through secondary tooth fluoride deposits. Prior studies by Armstrong (*18*) report an inhibitory effect "of extra fluorine," "when given in high concentration in drinking water for a period preceding the caries regimen." No analyses for fluorine are reported but the author believes the effect "was mediated by fluorine which had become incorporated in the dental enamel during the period of high fluorine consumption" (*17*). No epidemiological or clinical studies have been made of secondary fluoride as might be acquired by the adult human tooth.

EXPERIMENTAL

Three groups of rats aged 40 days, 100 days, and 200 days, respectively, were paired as litter mates. One rat of each pair received 100 p.p.m. of fluorine as sodium fluoride in its drinking water for specified periods (see table I). During this period the ration fed consisted of 66 percent corn flour, 30 percent whole milk powder, 3 percent alfalfa meal, and 1 percent salt. This fluoride period was followed by a 100 day period during which the rats were fed a caries producing diet. This diet was the same as the above diet except that coarse cornmeal was substituted for the corn flour. At all times food and water were consumed *ad libitum*. Caries was scored and recorded as in previous studies (*1, 2, 19*), being the system followed by Cox, Dodds, Dixon and Matuschak (*20*). The crowns of the molar teeth were pooled according to control or fluoride treatment. They were pulverized to pass a 60 mesh sieve, washed with alcohol and ether, and separated into dentin and enamel by the method of Manley and Hodge (*21*). Fluorine was determined (*22*) on the enamel and dentin, the results appearing in table II.

Among the 40-day-old rats all fluoride rats of the 14 pairs were affected with less caries than their nonfluoride pair mates. Their consistent favoring of the fluoride rat among these 14 trials can be regarded as due to the imposed fluoride factor. Likewise, the average data for carious teeth, carious areas and average caries score are strikingly lower for fluoride rats than for their control pairmates. Among the 10 pairs 100 days old at start, 7 paired results favored the fluoride rat, 1 of the pairs showed no difference, 2 results favored the control rat. The results for the 200-day-old rats were less significant; 6 results favored the fluoride rat, 2 pairs were the same, 3 favored the control rat. However, the averages for number of carious teeth and carious areas, and the average caries score all favored the fluoride rats in these two later groups.

In the 34 possible comparisons of control and fluoride rats, in terms of caries score, 28.5 cases favored the fluoride rat (where no difference between the two rats of a pair occurred, a value of 0.5 is allotted to each rat). The standard deviation of a distribution of 34 events which may occur with equal probability in either of two ways equals $\sqrt{0.5 \times 0.5 \times 34}$ or 2.92. The expected outcome in this series of events is 17 trials favoring the fluoride rat and 17 favoring the control. The difference between the expected result, 17.0, and the observed, 28.5, equals 11.5, a value 3.91 times the standard deviation, 2.92. An outcome such as this would occur fortuitously about once in 10,000 trials (*23*).

The results of fluorine analyses of molar dentin and enamel again show a retention of fluorine by mature rat dentin and enamel. Rats aged 40 days, 100 days, and 200 days show this property. The results for these teeth

Table I.—*Effect of fluoride ingested by rats prior to placing on caries-producing diet*

(Rats aged 40 days at start of fluoride—100 p.p.m. fluorine in drinking water for 85 days—prior to 100 days on caries producing diet)

Pair Number	1		2		3		4		5		6		7		8		9		10		11		12		13		14		Ave.	
Prior treatment	F	—	F	—	F	—	F	—	F	—	F	—	F	—	F	—	F	—	F	—	F	—	F	—	F	—	F	—	F	—
Carious teeth	2	3	0	3	0	3	2	3	0	2	1	3	1	4	1	3	0	2	0	2	0	1	0	3	3	3	0	4	.7	2.8
Carious areas	2	3	0	4	0	4	2	9	0	2	1	3	1	7	1	4	0	2	0	2	0	1	0	5	3	11	0	11	.7	4.9
Caries score	2	7	0	5	0	8	2	25	0	2	2	5	2	17	2	7	0	3	0	2	0	2	0	10	4	12	0	33	1.0	9.1

(Rats aged 100 days at start of fluoride—100 ppm. fluorine in drinking water for 60 days—prior to 100 days on caries producing diet)

Pair Number	15		16		17		18		19		20		21		22		23		24		Ave.	
Prior treatment	F	—	F	—	F	—	F	—	F	—	F	—	F	—	F	—	F	—	F	—	F	—
Carious teeth	2	3	0	4	6	4	0	4	0	1	3	2	1	3	4	4	1	1	4	4	2.1	3.0
Carious areas	4	9	0	16	17	4	0	9	0	1	3	2	1	9	8	10	1	1	4	12	3.8	8.2
Caries score	9	25	0	48	48	5	0	26	0	3	4	2	3	23	21	27	2	2	4	35	9.1	19.6

(Rats aged 200 days at start of fluoride—100 ppm. fluorine in drinking water for 40 days—prior to 100 days on caries producing diet)

Pair Number	25		26		27		28		29		30		31		32		33		34		Ave.	
Prior treatment	F	—	F	—	F	—	F	—	F	—	F	—	F	—	F	—	F	—	F	—	F	—
Carious teeth	4	3	3	4	0	4	0	0	3	2	0	0	0	3	1	0	4	3	2	3	1.7	2.2
Carious areas	6	12	5	2	0	13	0	0	3	4	0	0	0	3	1	0	14	3	2	3	3.1	4.0
Caries score	10	32	7	9	0	39	0	0	5	9	0	0	0	4	1	0	39	5	2	3	6.4	10.4

Table II.—*Assimilation of fluorine by mature rats' molar teeth*

	Enamel fluoride	Dentin fluoride
	per cent	*per cent*
40 day old rats given 100 ppm. fluoride in drinking water for 85 days, followed by 100 days without fluoride	.017	.128
Control rats (no fluoride)	.002	.005
100 day old rats given 100 ppm. fluoride in drinking water for 60 days, followed by 100 days without fluoride	.014	.057
Control rats (no fluoride)	.004	.005
200 day old rats given 100 ppm. fluoride in drinking water for 40 days, followed by 100 days without fluoride	.016	.048
Control rats (no fluoride)	.009	.008

were obtained after the fluoride regimen had been stopped for 100 days. Presumably these figures represent total fluoride deposited during the fluoride period. The actual stability of fluoride deposits in dental tissue is unknown, data based on rats (*24*) and humans (*25*) having indicated that fluorine deposited in the body may be mobilized from body tissue (presumably mostly bone) and excreted in the urine. The stability of calcium and phosphorus in the dental tissues would seem to indicate that fluorine once acquired by dentin and enamel remains as a permanent deposit. It should be emphasized that the differences in fluoride retained by teeth of rats of different age, can not be ascribed to the rats' age, since no attempt was made to equalize the quantity of fluoride ingested by these rats. These analytical results merely show again the capacity of the adult rat tooth to retain fluorine. A suggestion has been made, however, that "fluorine retention may prove a useful tool in studying individual variations in dentin and enamel in relation to tooth age and susceptibility to dental caries" (*3*).

DISCUSSION

Previous studies (*1, 2*) showed that fluorine may be deposited posteruptively in rats' teeth. However, these experiments as conducted (*1*) did not make it possible to discriminate between caries inhibitory effects of fluoride in the diet or drinking water, and fluoride acquired by the dental tissue during posteruptive tooth life. Also, in an injection experiment (*2*) the observed negative effect of fluoride as found in the enamel and dentin may have been due to insufficient tooth fluoride being acquired, especially in the outer surface enamel during the early part of the caries inducing period. The results of the present study seem to justify the conclusion that 40-day-old rats given a preperiod of fluoride during posteruptive tooth life show a definitely decreased incidence and severity of induced caries. It seems logical to conclude that caries was inhibited by fluoride, which had been incorporated in the adult dentin and enamel during the period of fluoride ingestion. There is evidence, therefore, that not only will mature rat dentin and enamel retain ingested fluoride as a secondary deposit, but that such fluoride may increase caries resistance. The less significant effect of fluoride in the older rats may be due to the fact that less fluorine was acquired by their dentin and enamel (see table II).

The evidence that fluorine may penetrate the exposed oral enamel is lacking in these results, but there is also no evidence that this phenomenon does not occur. Actually, the rat is unsatisfactory for a study of oral enamel phenomena exclusively, since the occlusal surface of the rat's molar has some exposed dentin, which would be expected to retain fluorine to a similar if not greater extent than enamel.

The observed adsorption of fluorine by mineral phosphates (*8, 9, 10*), bone (*7*), and dental tissues (*5, 6*) is only presumptive evidence that exposed enamel in the oral cavity has a similar capacity. In general, the fluorosed phosphates and bone and dental tissue resulting from these reactions show reduced acid solubility (*6, 9* and unpublished data from this laboratory). Artificially fluorosed enamel and dentin and also pure inorganic fluorides also appear to reduce bacterial acid production (*26, 27*). Such acquired properties suggest an inhibitory effect on fluorosed dental tissues against dental caries. While it is known that primary fluoride in the teeth is associated with mottled enamel, in quantities equaling 0.025 per cent to 0.036 percent of the enamel (*28*), this fluoride is acquired and, therefore, must be ingested during tooth formation (*29*). Fluoride ingested following tooth eruption does not produce mottling in human teeth (*28*) nor in rats' molar teeth (*1*). Adsorbed secondary enamel fluoride would not be expected to cause mottled enamel.

It is scarcely necessary to point out that much more information is needed as regards both the actual adsorption of fluorine by enamel, in situ, and the caries inhibitory or other effects of such fluoride should it be adsorbed.

SUMMARY

Rats aged 40 days, 100 days, and 200 days were given fluoride prior to placing them on a caries producing diet. There was strong evidence that this prefluoride period brought about an appreciable resistance to rat caries particularly in the youngest rats. Since fluoride was found to have increased notably in the dentin and enamel of the rats' teeth, the effect seems to be due to fluoride incorporated in the adult tooth. It seems reasonable to expect, in the light of these results, that should the adult human tooth be made to acquire fluorine similarly, as a secondary deposit, it might thereby be partially protected against dental caries.

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COMPLEX FLUORIDES: CARIES REDUCTION AND FLUORINE RETENTION IN THE BONES AND TEETH OF WHITE RATS

By I. ZIPKIN and F. J. McCLURE

This study was made to obtain data on the physiological availability and effects of fluorine as it occurs in several complex chemical combinations. The criteria for evaluation of these fluorides (as administered by injection and in drinking water of growing rats) were (*a*) deposition of fluorine in bones and teeth, (*b*) development of incisor striations and, (*c*) ability to inhibit experimental rat caries. These and similar experiments not only help to elucidate the metabolism of fluorine but may also have a practical application to fluoridation of drinking water, which has become an effective procedure for the partial control of human dental caries. Although sodium fluoride (NaF) is now the most common fluoride in use for community water fluoridation, other compounds, particularly sodium fluosilicate (Na_2SiF_6), if found comparable to NaF in physiological effects, may have an advantage in being produced at less expense than NaF. Since commercial grade NaF is 95 percent pure, and at current market quotations (*21*) sells for 11 cents per pound, and commercial Na_2SiF_6 is 98 percent pure and sells for 5 cents per pound, the cost per pound of available fluorine is now approximately three times more for NaF than for Na_2SiF_6.

In a previous publication (*1*) the junior author reviewed earlier reports of the effects of a number of different fluorides (NaF, Na_2SiF_6, Na_3AlF_6, $BaSiF_6$ NH_4F, KF, K_2SiF_6, and CaF_2) and reported that NaF and Na_2SiF_6, when ingested in drinking water by growing rats in amounts which furnished 5, 10, 15, 25, and 50 p.p.m. of fluorine, resulted in equivalent retentions of fluorine in the bones and teeth, mandibles, and femurs, and produced similar incisor striations. In general, this study (*1*) indicated that fluorine in NaF and Na_2SiF_6 is equally available.

Deposition of fluorine in dental and skeletal tissues of hamsters receiving Na_3AlF_6, Na_2PO_3F, KPF_6, NaF, and "Flural" (*2*) was also recently reported. Flural is a commercial preparation of the Ozark-Mahoning Company, Tulsa, Okla., containing alum and a fluoride. One commercial grade has a composition approximating the formula $AlFSO_4 \cdot 2H_2O$ (*6*). Less fluorine was retained in the lower incisors of hamsters receiving "Flural", KPF_6, and Na_3AlF_6, than in hamsters receiving NaF, but fluorine retained in the tibias was proportional to the fluorine ingested, regardless of the type of fluoride fed (*2*).

Data on the biological availability of covalent-bonded fluorine are very meager. Kempf et al. (*3, 4*) reported that α-fluoronaphthalene produced mottled enamel, whereas p, p-difluro-diphenyl, p-fluorobenzoic acid and fluorobenzene had no such effect. No tissue analyses for fluorine were reported. Boyer et al. (*5*) did not find any increase of fluorine in the bones of rats fed 0.004 percent 3-fluorotyrosine in the diet; in fact, less fluorine was found than in control rats receiving no fluoride. Euler and Eichler (7), however, reported that 2 mg./kg. of 3-fluorotyrosine given by stomach tube produced mottling in rat incisors and histological changes in the bones and teeth. No tissue analyses for fluorine were reported. Armstrong et al. (*8*) recently have reported "bleaching of the teeth" and the presence of inorganic fluoride in the urine of rats receiving 4-fluorophenylalanine by mouth. Hagen et al. (*9*) have also presented evidence that sodium monofluoroacetate may be metabolized by the rat, since only 22 percent of the sodium monofluoroacetate fed could be recovered in the carcass and excreta as the parent compound.

One significant criterion of the physiological availability of different fluorine compounds is their effect on experimental animal caries. Comparable evidence is somewhat meager in this regard, since the majority of caries inhibition studies relate to the effects of sodium fluoride. Keyes and Shourie (*10*) reported that 50 p.p.m. fluorine as sodium fluoride "was very effective in reducing caries activity in the molar teeth of hamsters, sodium fluosilicate was somewhat less effective, and calcium fluoride was essentially ineffective." More recently, the acute toxicity and ability to inhibit caries in hamsters were used as criteria for a comparison of NaF and Na_2PO_3F (*11*). Comparable caries reduction was attributed to Na_2PO_3F and NaF when administered in the drinking fluid at a level of 40 p.p.m. F. However, on the basis of fluorine content, the complex fluoride Na_2PO_3F appeared to be 2.5 to 3.0 times less toxic than NaF (*11*). In further studies, this group of workers reported that Na_3AlF_6, "Flural", Na_2PO_3F, and NaF had "nearly maximal" inhibitory effects on hamster caries, but that KPF_6 did not reduce caries significantly (*2*).

It is generally true that the majority of the fluorine compounds studied up to this time, when ingested at levels just sufficient to produce characteristic striation in rats' incisor teeth, have similar effects. At higher intakes, differences in physiological effects generally have been attributed to different solubilities which seemingly affect absorption from the digestive tract. Aside from the factors of solubility and concentration, however, fluorine in some chemical compounds, even though adequately soluble and absorbed from the digestive tract may not be metabolized. Such fluorides would not be expected to show characteristic effects of the fluoride ion per se, and would not deposit fluorine in body tissues. Thus, Boyer et al. (*5*), as noted above, reported that no fluoride could be found in rats fed 3-fluorotyrosine in the diet. Two inorganic fluorides, Al_2F_6 (*3, 4*) and ZnF_2 (*3*), have also been reported not to produce incisor striations when fed in the diet at a concentration of 0.10 percent F. From our experiments reported below, it now appears that two other fluorides, KPF_6 and CF_3-COONa, although highly soluble, do not produce characteristic fluorine effects and cause no deposition of fluorine in body tissues.

EXPERIMENTAL

One hundred and fifty female white rats of the Holtzman strain, 21–27 days of age, representing 30 litters of five rats each, were equally distributed into five groups. An additional 180 males of the Holtzman strain, 21–27 days of age, representing 30 litters of six rats each, were distributed equally into six groups All rats received, *ad libitum*, a cariogenic diet of the following composition:

	Percent
Whole milk powder	30.0
Yellow corn grits	42.0
Cane sugar, granulated	25.0
Whole dried liver substance (Wilson)	2.0
Salt mixture	1.0

Salt mixture	*Grams*
Sodium chloride	400
Potassium chloride	400
Magnesium carbonate	100
Iron and ammonium citrate	20
Manganous sulfate	28
Calcium hydrogen phosphate	50
Cupric acetate	2

The plan of the experiment was as follows:

Group	*Number rats*	*Sex*	*Fluid*	*Route of administration*
Control	30	F	Distilled water	Peroral.
Drink B	30	F	50 ppm F as Na_2SiF_6	Peroral.
Inject B	30	F	500 ppm F as Na_2SiF_6	I. P.
Drink C	30	F	50 ppm F as Na_2PO_3F	Peroral.
Inject C	30	F	500 ppm F as Na_2PO_3F	I. P.
Control	30	M	Distilled water	Peroral.
Drink D	30	M	50 ppm F as KPF_6	Peroral.
Inject D	30	M	500 ppm F as KPF_6	I. P.
Drink E	30	M	50 ppm F as NaF	Peroral.
Inject E	30	M	500 ppm F as NaF	I. P.
Drink FF	30	M	50 ppm F as CF_3COONa	Peroral.

All groups received approximately the same quantity of fluorine, except the rats receiving Na_2SiF_6 injected intraperitoneally. Injected rats received 3.5 mg. fluorine weekly, distributed over five daily injections. Because of its high acidity (pH 3.3), the inject Na_2SiF_6 solution was poorly tolerated; for this reason these rats received less fluoride, since the study was terminated when all the rats had been on the cariogenic diet the same length of time. The diet contained 1.3 p.p.m. F, which was considered negligible in calculating the total fluorine consumption.

At the end of 91 days, the animals were killed and the teeth diagnosed for dental caries according to Cox et al. (*12*). The femurs, mandibles, molars, and incisor teeth were dried, extracted with alcohol and ether and ground to pass a 60-mesh sieve. The incisors and molars were separated into dentin and enamel by the Manly-Hodge technique (*13*). All tissues were then ashed at 550° C. for 3 hours and analyzed for total fluoride (*14*).

ANALYSIS OF FLUORINE COMPOUNDS

The preparation of the fluoride solutions required a careful assay for purity and for free fluorine in KPF_6, Na_2PO_3F, and CF_3COONa, which were commercial grade samples. NaF and Na_2SiF_6, being analytical grade reagents, were accepted according to specifications. Na_2PO_3F was found to contain 13.5 percent total fluorine (*14*) (13.2 percent theory), and 1.85 percent free fluorine (*15*), indicating that 13.7 percent of the total fluorine was present as free fluorine. This commercial product specified a purity of 90–97 percent with NaF, $(NaPO_3)_x$, and Na_2CO_3 as impurities. By repeated shaking of solutions of this commercial Na_2PO_3F with MgO, free fluorine was reduced to 7.0 percent of the total fluorine. This purified Na_2PO_3F solution was used to prepare the rat's drinking and injection solutions.

KPF_6 contained no free fluorine (*16*) and averaged 94.6 percent purity by the Willard and Winter fluorine analysis (*14*) and 98.6 percent purity by the PbClF precipitation procedure (*17*). Similarly, no free fluorine was found in CF_3COONa. However, analysis of CF_3COONa for total fluorine by the usual Willard and Winter perchloric acid distillation was unsatisfactory, no fluorine being detected in the distillate by $Th(NO_3)_4$ titration. Ashing of CF_3COONa for 3 hours at 550° C. in the presence of CaO gave only 65.9 percent recovery of theoretical fluorine. Further analysis of CF_3COONa by fusion with Na_2CO_3 followed by precipitation as PbClF (*17*) gave 92.0 percent of theoretical fluorine. The most satisfactory analysis was obtained by fusing 95.4 mg. CF_3COONa, 15 gm. Na_2O_2, and 0.4 gm. sucrose in the Parr bomb, dissolving the fused mixture in hot water, neutralizing with HCl, and diluting to 2 liters with distilled water. This solution when titrated for fluorine with $Th(NO_3)_4$ gave 98.0 percent of theoretical recovery, which agrees well with the reported assay of 98–99 percent.

RESULTS OF EXPERIMENTS

Without exception all the fluorine compounds, when injected, caused no reduction in dental caries (see table). Though this negative result cannot be explained by the absence of fluorine in the molar dentin and enamel of these rats, it is nonetheless possible that fluorine as acquired by these teeth from parenterally administered fluoride did not reach the oral enamel surface in sufficient time perhaps, or in sufficient quantity, to exert a cariostatic effect. It will be noted that the quantity of fluorine in molar enamel of injected rats is consistently lower than the fluorine in the molar enamel of rats receiving fluorides orally. This negative effect of injected fluoride agrees with previous results with rats (*18*) and, to some extent, with previous results with hamsters (*10*). Although hamster caries appeared to be inhibited by injected fluoride, it was suggested that fluorine may have reached the oral cavity via coprophagy.

Also, no caries inhibition occurred with orally administered KPF_6, which agrees with a previous hamster study (*2*), nor from oral CF_3COONa. The absence of any increased fluorine in the teeth of these rats and the lack of enamel striations are consistent with this observation. Reduction of caries was similar in the three groups of rats given NaF, Na_2SiF_6, and Na_2PO_3F in their drinking water. The teeth of these rats responded also by a greatly increased fluorine content in the enamel and dentin. The caries reducing effect of NaF has been repeatedly demonstrated (*19*), and the caries inhibiting effect of Na_2SiF_6 was anticipated by evidence of the availability of its fluorine (*1*). As previously mentioned, other studies (*11*) have also shown the ability of Na_2PO_3F to reduce caries in hamsters. Reduction in the percent of rats having caries, i.e., the reduced incidence of caries, was 21.3, 19.4, and 15.7 percent, respectively, for NaF, Na_2SiF_6, and Na_2PO_3F. Severity of caries as indicated by the caries score was reduced 39.2 percent by NaF, 45.0 percent by Na_2SiF_6, and 45.0 percent by Na_2PO_3F. These three fluorides, therefore, were essentially similar in their cariostatic effects.

As shown in the table, two groups of rats, one male, the other female, served as controls. Since differences in caries between these two groups were not statistically significant, they were combined to give a composite control group which was used as the standard of reference. This result thus contributes to the evidence that sex is probably not a factor in the production of rat caries.

The complex fluorides KPF_6 and CF_3COONa were strikingly different from the other fluorides in the physiological availability of their fluorine. No fluorine was deposited in the bones and teeth of rats ingesting these compounds. The data are graphically presented on the chart.

Owing to difficulties in the fluorine analysis of CF_3COONa, some uncertainty arose as to the failure to find fluorine in bones and teeth of rats receiving this compound. The remote possibility existed that fluorine could be deposited as trifluoroacetate or some metabolic intermediate, which would not be detected by the Willard and Winter procedure (*14*). Thus, when standard solutions of CF_3COONa were added to fresh bone samples, which were then ashed and distilled in the usual procedure (*14*), no fluorine was found in the distillate. However, CF_3COONa added to ashed bone resulted in 62.4 percent of fluorine recovery, indicating some interference due to the organic matrix of

Metabolism and caries inhibitory effects of fluorine in different chemical combinations

Item	Control	Control	Control	NaF oral	NaF inj.	Na_2SiF_6 oral	Na_2SiF_6 inj.	Na_2PO_3F oral	Na_2PO_3F inj.	KPF_6 oral	KPF_6 inj.	CF_3COONa oral
	Male	*Female*	*Both*	*Male*	*Male*	*Female*	*Female*	*Female*	*Female*	*Male*	*Male*	*Male*
Total fluorine (mg.)	0.0	0.0	0.0	62.6	60.5	50.5	46.7	61.2	57.2	64.2	60.6	63.0
Days on experiment	92	92	92	91	91	91	91	91	92	91	91	91
Final weight (gm.)	260	187	222	275	247	195	178	187	189	280	255	275
Initial weight (gm.)	35	35	35	33	35	36	38	36	37	35	35	34
Average daily gain (gm.)	2.4	1.7	2.0	2.7	2.3	1.7	1.5	1.7	1.7	2.7	2.4	2.6
	Caries diagnosis											
Number of rats	27	30	57	29	23	27	29	27	27	27	26	27
Number of litters	27	30	57	29	23	27	29	27	27	27	26	27
Rats with caries (number)	27	28	55	22	23	21	28	22	25	26	26	26
Rats with caries (percent)	100.0	93.3	96.5	75.9	100.0	77.8	96.6	81.5	92.6	96.3	100.0	96.3
Carious teeth per carious rat (number)	4.1	3.5	3.9	3.6	4.7	2.8	3.9	3.0	4.0	4.4	4.3	4.2
Carious teeth per carious rat (percent)	34.2	29.2	32.5	30.0	39.2	23.3	32.5	25.0	33.3	36.7	35.8	35.0
Carious areas per carious rat (number)	6.5	5.3	5.9	3.7	7.3	3.2	6.5	3.4	5.7	7.2	8.0	6.4
Caries score per carious rat	13.2	10.8	12.0	7.3	15.0	6.6	14.6	6.6	13.4	15.4	17.7	12.8
	Fluorine analysis of teeth and bones (ash basis)											
Molar enamel (percent)	0.005	----------	[1](0.005)	0.053	0.036	0.071	0.022	0.059	0.029	0.001	----------	0.003
Molar dentin (percent)	.004	0.011	(.008)	.233	.280	.216	.224	.249	.255	.001	0.006	.00[illegible]
Incisor enamel (percent)	.003	.005	(.004)	.044	.053	.053	.057	.053	.064	.001	.004	.012
Incisor dentin (percent)	.004	.008	(.007)	.244	.256	.213	.336	.266	.284	.001	.004	.007
Mandible (percent)	.009	.006	(.007)	.263	.324	.476	.528	.533	.520	.003	.006	.010
Mandible total (mg.)	.01	.02	(.02)	1.2	1.1	1.3	1.1	1.3	1.1	.01	.01	.03
Femur (percent)	.004	.009	(.007)	.419	.486	.497	.488	.555	.619	.006	.007	.012
Femur total (mg.)	.04	[illegible]	(.03)	2.2	2.2	2.4	2.0	2.3	2.3	.03	.03	.06
	Percent ash content of teeth and bones											
Molar enamel	94.8	[illegible]	[1](94.7)	94.2	94.4	94.6	94.4	94.4	94.3	94.2	94.4	94.2
Molar dentin	75.8	[illegible]	(75.3)	76.9	77.1	77.6	76.8	77.0	77.4	76.4	77.1	75.7
Incisor enamel	95.6	[illegible]	(95.4)	94.8	94.5	94.8	95.2	94.5	95.2	94.8	94.9	94.6
Incisor dentin	75.3	[illegible]	(75.5)	73.4	75.0	75.0	75.6	75.1	74.9	74.6	75.1	75.3
Mandible	69.9	[illegible]	(70.2)	71.5	71.0	72.1	72.3	72.2	70.9	70.2	69.8	70.8
Femur	69.7	64.9	(67.3)	68.1	66.5	68.4	70.5	70.8	66.2	66.4	66.9	68.6
Enamel striations	(2)	(2)	(2)	(3)	(3)	(3)	(3)	(3)	(3)	(2)	(2)	(2)

[1] Arithmetic mean of male and female control rats. [2] None. [3] Marked.

skeletal tissues. The recovery of fluorine added as CF_3COONa to bone could be improved by a preliminary distillation of the fresh bone plus CF_3-COONa, using H_2SO_4 at 160° to 165° C., followed by a second distillation using $HClO_4$ (*15*); 81.1 percent of the CF_3-COONa was recovered by this procedure. Bone samples alone analyzed by this technique gave no fluorine over and above that obtained by the usual $HClO_4$ distillation.

It seems reasonable to assume, therefore, that no fluorine, free or combined, was deposited in these animals' skeletal or dental tissues. Availability of fluorine in NaF, Na_2SiF_6, and Na_2PO_3F, however, was similar and pronounced, as indicated by the high fluorine content of the skeletal and dental tissues of rats exposed to these fluorides. The similarity in availability of fluorine in NaF and Na_2SiF_6 was previously shown by McClure (*1*) and the availability of fluorine in Na_2PO_3F as fed to hamsters was also previously reported (*11*). It is of interest to note the similarity of the fluorine deposits in molar dentin and incisor dentin. Although the incisor is actively growing as compared with the fully developed molar, both have retained similar quantities of fluorine in the dentin.

Differences in skeletal fluorine deposition from oral vs. injected NaF, Na_2SiF_6, and Na_2PO_3F were found not to be statistically significant according to Fisher's "t" test (*20*). The same holds true for fluorine deposition in dental tissues with the exception of molar enamel. As previously noted, more fluorine is present in the molar enamel of rats receiving NaF, Na_2SiF_6, and Na_2PO_3F orally than by injection. This difference may be due to adsorption of fluorine on the enamel surface from orally administered fluoride. As also noted above, this may account to some extent for the inability of injected fluorides to inhibit caries.

Data for the ash content of the bones and teeth are similar for all groups and are indicative that no quantitative changes in calcification may be attributed to these various fluoride compounds. By comparison with control rats, the average daily gain in weight was not affected by any of the fluorides fed.

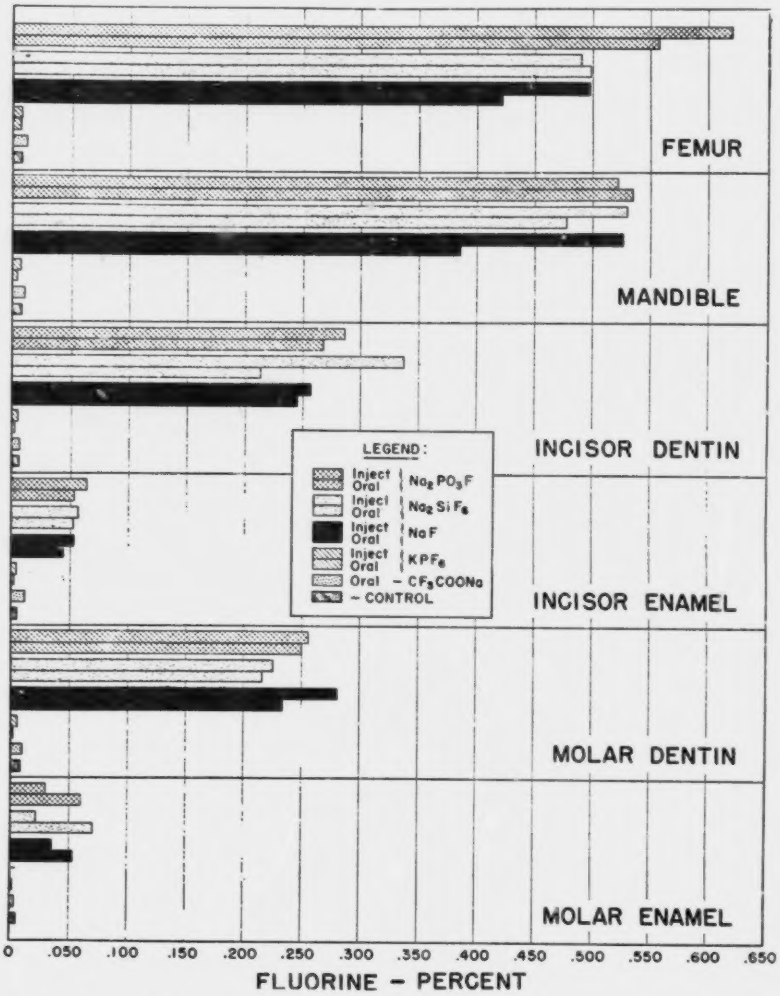
DISCUSSION

The observed cariostatic effects of NaF and Na_2SiF_6 extend our previous evidence of a similar physiological availability of the fluorine in these two compounds (*1*) and would appear to further justify the use of Na_2SiF_6 for domestic water fluoridation. The close physiological identity of these two compounds is not surprising as both compounds liberate fluoride ions in dilute aqueous solutions.

The effects of Na_2PO_3F paralleled those of NaF and Na_2SiF_6 in all regards. Our results thus point to an effect of the fluoride ion, per se, liberated during the metabolism of Na_2PO_3F. However, Shourie et al. (*11*) suggest that fluorine does not have to exist as the free ion to inhibit caries. These investigators' supposition that Na_2PO_3F is not hydrolyzed in the body is, at present, based on indirect evidence that Na_2PO_3F, both orally and parenterally administered in single doses, is 2.5 to 3 times less toxic that NaF on the basis of fluorine content. Further studies seem to be in order to clarify the *in vivo* hydrolysis of Na_2-PO_3F. The data of our experiments support the belief that Na_2PO_3F is metabolized by the rat in a manner similar to NaF and Na_2SiF_6, since all these compounds had similar effects on dental caries, enamel striations, and storage of skeletal and dental tissue fluorine. This belief is also supported by the statement that Na_2PO_3F slowly hydrolyzes in acid solution to give fluoride ions.

Viewed as a basic problem in the metabolism of fluorine, the results obtained with KPF_6 and CF_3COONa are perhaps the most interesting outcome of this study. Fluorine in these combinations seems to be totally unavailable to

Fluorine in ash of bones and teeth of rats receiving fluorine as NaF, Na_2SiF_6, Na_2PO_3F, KPF_6, *and* CF_3COONa



the rat when administered either orally or parenterally.

There seems to be a relation of "saturation" of fluorine in complex fluorides (perfluoro compounds) to the physiological stability of fluorine. Thus, sodium monofluoroacetate appears to be metabolized to furnish inorganic fluoride (*9*), whereas sodium perfluoroacetate (CF_3COONa) does not yield inorganic fluoride. Similarly, sodium monofluorophosphate is metabolized, whereas potassium perfluorophosphate (KPF_6) does not appear to be hydrolyzed by the rat. It would seem, therefore, that the metabolism of simple and complex fluorides does not follow a similar pattern, and more evidence is needed to elucidate these differences.

SUMMARY

A comparative study of the physiological effects of NaF, Na_2SiF_6, KPF_6, Na_2PO_3F, and CF_3COONa was made using the young growing rat as the experimental animal. Fluorine was ingested in the drinking water at a level of 50 p.p.m. and, with the exception of CF_3COONa, all the compounds were also injected intraperitoneally. NaF, Na_2SiF_6, and Na_2PO_3F in the drinking water reduced dental caries, deposited fluorine in the bones and teeth, and caused marked incisor striations to essentially the same extent. None of the fluorides had any cariostatic effect when administered by intraperitoneal injection. However, injected NaF, Na_2SiF_6, and Na_2PO_3F produced enamel striations and deposited fluorine in the bones and teeth. KPF_6 and CF_3COONa were physiologically inert insofar as could be indicated by caries inhibition, enamel striations, and deposition of fluorine in bones and teeth.

The data suggest that NaF, Na_2SiF_6, and Na_2PO_3F may be equally effective as water fluoridating agents for caries prevention.

ACKNOWLEDGMENTS

We are indebted to Chemist H. G. McCann of the National Institute of Dental Research for the purification of Na_2PO_3F and for the fluorine analysis of KPF_6 and CF_3COONa by the PbClF precipitation procedure.

We are also indebted to the Ozark-Mahoning Company, Tulsa, Okla., for Na_2PO_3F and KPF_6 and to the Hooker Electrochemical Company, Niagara Falls, N.Y., for CF_3COONa.

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SMOOTH SURFACE CARIES IN WHITE RATS: EFFECTS OF FLUORIDE, IODOACETATE, PENICILLIN, CRISCO, BUTTERFAT AND A SALT MIXTURE

F. J. McClure, J. E. Folk, and John D. Rust

The results of experiments on smooth surface caries in white rats have been previously reported (*1–7*). Studies are now in progress to determine what components and properties of the diet may be involved in the development of this type of caries and what agents or conditions promote or inhibit its incidence and severity.

In a recent symposium, the effects of metallic and nonmetallic compounds, organic compounds, fluoride compounds and antibiotic agents on experimental caries were discussed (*8*). There is consistent and substantial evidence that fluoride, iodoacetate and penicillin have a considerable preventive effect on experimental rat caries generally classified as the occlusal fissure type (*8*). There are other reports that penicillin, Terramycin (oxytetracycline), a salt mixture and Wesson oil were preventive toward smooth surface caries (*8*). Corn oil, olive oil, Wesson oil, Crisco and lard were reported to be preventive toward fissure caries in rats (*9*). Butterfat, according to another study, had no effect on fissure caries (*10*). A 35 percent level of fat, mostly lard, was credited with having a considerable inhibitory effect on fissure caries in the cotton rat, according to results based on two to six animals per group (*11*). Inhibition of hamster caries has been related to dietary supplements of arachis oil, hydrogenated arachis oil and lard (*12*), and also cocoa fat, olive oil and poppy seed oil (*13*).

The data of this study pertain to the posteruptive effects of fluoride, penicillin, iodoacetate, butterfat, Crisco and Osborne-Mendel salts, respectively, on the smooth surface caries associated with National Institute of Dental Research (NIDR) diet 636 (*3*) and also the effect of butterfat on this same type of caries as associated with NIDR diet 635 (*3*).

METHOD

The evaluation of caries produced in these experiments and the preparation of the diets are described elsewhere (*3, 6*). Diet 635 contained 35 percent of a commercial roller-processed skim milk powder, 45 percent cornstarch, 18 percent glucose and 2 percent liver powder. Diet 636 was the same as diet 635 except that the skim milk powder was "dry autoclaved" (*3*). The majority of the carious lesions occur on lower buccal surfaces and bear a close resemblance to gingival caries in human beings (fig. 1). Two strains of rats, Holtzman and Sprague-Dawley, were employed in each experiment. The rats were started on experiment at weaning age, were housed two per cage and were on the diets for approximately 90 days. Littermates were used to compare control with modified diets. Both sexes were represented in all comparable groups. The general management and plan of the studies are indicated in table 1, in which the data are presented.

The effects of sodium fluoride and iodoacetate were evaluated in two series of experiments using diet 636 (table 1, experiment 1-a, 1-b and 1-c and 2-a through 2-e). The effect of penicillin (L-ephenamine penicillin G) (*14*) was evaluated in experiment 2-f and 2-g. The penicillin diet was renewed every day; the other diets, 5 days per week. Feeding was ad libitum.

One series of experiments (3-a, 3-b and 3-c) determined the effect of 13 percent butterfat and 13 percent Crisco added to diet 636. In another series (4-a, 4-b, 4-c and 4-d), 10 percent butterfat was added to diets 635 and 636. The butterfat and Crisco replaced an equivalent amount of cornstarch in the diet. For experiment 4-d, butterfat was added to the same skim milk powder used in diet 635 (4-a) and the mixture "dry autoclaved" before using it to prepare diet 636. In this way it was possible to evaluate the effect of a prior heat treatment on this mixture of butterfat and skim milk powder.

Experiments 5-a and 5-b were designed to study the effect of adding 4 percent Osborne-Mendel salts, (*15*) with sodium fluoride omitted, to diet 636 in place of 4 percent of the cornstarch. Without these salts, the diet contained 2.72 percent ash, 0.42 percent calcium and 0.38 percent phosphorus. Practically all the minerals of diet 636 are provided by the 35 percent skim milk powder and 2.0 percent dried liver. Diet 636 with added Osborne-Mendel salts contained 5.69 percent ash, 0.93 percent calcium and 0.66 percent phosphorus.

RESULTS

Effect of Fluoride, Iodoacetate and Penicillin on Smooth Surface Caries.—The data obtained in these studies appear in table 1. The carious lesions are illustrated in figure 1.

Fluoride, iodoacetate and penicillin were consistently preventive toward caries when added to diet 636. The effect of fluoride was demonstrated by a pronounced difference between the control and experimental groups in caries incidence (percentage of the rats having caries), in the number of carious teeth per rat and in the caries score which is used to evaluate relative severity (3). The data for the two levels of fluoride (experiment 2-b and 2-c) suggest a slightly greater effect at the higher level of fluoride, a result comparable to the relative effects of increasing quantities of fluoride on occlusal surface caries (16).

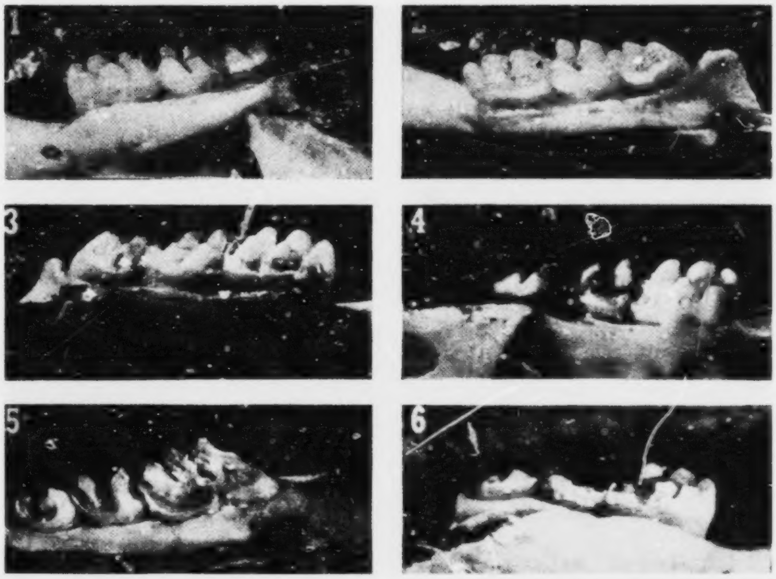

FIGURE 1.—*Carious lesions. 1, 2: Initial carious lesions on lower buccal surfaces. 3: Progress of caries particularly as it follows line of gingival attachment. 4, 5: Carious lesions involved dentin and much of buccal surface. 6: Crowns of second and third lower molars were destroyed. (Illustration from McClure, F. J. Dietary factors in experimental rat caries. In Advances in experimental caries research. Sognnaes, R. F., editor, Washington, D.C., American Association for the Advancement of Science, 1955)*

TABLE 1.—*Effect of sodium fluoride, iodoacetate, penicillin, butterfat, Crisco, and Osborne-Mendel salts on smooth surface caries in rats*

Exp	Supplements present in diet or drinking water	No. of rats	Daily gain (Gm.)	Rats with caries (%)	Carious teeth per rat (Av.)	Severity score per rat (Av.)
	Effect of fluoride, iodoacetate and penicillin in diet 636					
1-a	Unsupplemented	35	0	82.9	4.9	14.6
1-b	Sodium fluoride—50 ppm F in water	33	0	21.2	0.4	0.7
1-c	Iodoacetate—20 ppm in diet; 200 ppm in water	33	0.2	18.2	0.4	0.5
2-a	Unsupplemented	28	0.8	89.3	3.8	8.4
2-b	Sodium fluoride—25 ppm F in water	13	0.8	46.2	0.5	0.9
2-c	Sodium fluoride—50 ppm F in water	13	0.9	15.4	0.3	0.9
2-d	Iodoacetate—50 ppm in diet; 10 ppm in water	12	0.8	66.7	1.6	2.4
2-e	Iodoacetate—100 ppm in diet; 20 ppm in water	12	0.6	50.0	1.2	2.2
2-f	Penicillin—5 units per Gm. in diet	13	0.8	30.8	0.6	0.7
2-g	Penicillin—10 units per Gm. in diet	13	0.9	15.3	0.2	0.3
	Effect of butterfat and Crisco in diets 635 and 636					
3-a	Unsupplemented—diet 636	40	0.6	95.0	2.9	8.4
3-b	Butterfat in diet 636—13%	37	0.4	77.8	1.9	4.3
3-c	Crisco in diet 636—13%	38	0.4	64.9	1.2	2.0
4-a	Unsupplemented—diet 635	40	1.9	75.0	2.2	4.8
4-b	Butterfat in diet 635—10%	37	1.9	35.1	1.0	1.6
4-c	Butterfat in diet 636—10%	38	0.8	71.1	2.0	3.7
4-d	*Butterfat in diet 636—10%	33	0.6	72.8	2.3	4.4
	Effect of Osborne-Mendel salts in diet 636					
5-a	Unsupplemented	38	1.1	89.5	2.8	7.8
5-b	Osborne-Mendel salts in diet—4%	35	1.2	48.6	1.1	2.5

*Butterfat was autoclaved with milk powder.

Iodoacetic acid also was preventive toward smooth surface caries. When iodoacetic acid was added at different levels to the diet and drinking water, the highest amount in the diet and water combined (1-c in table 1) had the most pronounced preventive effect. In this study (1-c), the difference between the control and experimental group in caries incidence was 78.0 percent, in the number of carious teeth per rat, 91.8 percent, and in the caries severity score, 96.6 percent.

Penicillin at a level of 10 units per gram of diet (experiment 2-g) appeared to be considerably more preventive than at 5 units per gram (2-f). The small additions of penicillin to diet 636 caused a significant difference between the control and experimental groups in caries incidence, number of carious teeth per rat and caries severity score. The results for experiments 2-a, 2-c, 2-e and 2-g appear graphically in figure 2.

Effect of Butterfat and Crisco on Smooth Surface Caries. — Statistical analysis was made of severity scores and caries incidence. Severity scores were analyzed according to Fisher's "t" test of paired values. Difference in incidence was analyzed on a paired basis by the chi square test, $p > 0.05$.

This statistical analysis indicates that the difference in caries severity and the difference in caries incidence between the group receiving diet 636 unsupplemented and the group receiving diet 636 supplemented with Crisco were significant (experiment 3-a and 3-c). Butterfat added to diet 636 (3-b), however, did not change the caries incidence significantly, according to a statistical analysis of the data, and the difference in severity had only borderline statistical significance. A comparison of the data from experiment 4-a with that from 4-b showed a statistically significant effect of butterfat added at a 10 percent level to diet 635. There was a difference between the control and experimental groups in caries incidence of 53.2 percent, in the number of carious teeth per rat of 54.5 percent and in the caries severity score of 66.7 percent. Previous studies, as well as results obtained in these experiments, indicate that diet 636 is generally more cariogenic than diet 635; this fact might account for the difference in the caries preventive effect of butterfat in the two diets.

Butterfat autoclaved with the skim milk powder of diet 636 apparently was comparable to butterfat added to diet 636 without the autoclaving treatment. As shown by the results in experiment 4-c and 4-d, caries experience was about the same in both these groups. The effect of butterfat and Crisco in diet 636 is shown graphically in figure 3.

Effect of Osborne-Mendel Salts on Smooth Surface Caries.—The Osborne-Mendel salt mixture modified the cariogenic property of diet 636 to a rather remarkable extent (table 1). There was a difference between the control and experimental groups in caries incidence of 45.7 percent, in the number of carious teeth, 60.7 percent and in the caries severity score, 67.9 percent. These differences were statistically significant. These caries data are shown graphically in figure 4.

Inspection of the data in table 2 shows no apparent effect of the Osborne-Mendel salt supplement on the ash, calcium and phosphorus content of the molar dentin and enamel.

DISCUSSION

The exact mechanisms by which the incidence and severity of this experimental smooth surface rat caries was affected posteruptively by fluoride, iodoacetate and penicillin are not known. It has been established that fluoride is incorporated posteruptively into rats' teeth, (*16*) and resistance to caries possibly is dependent on the addition of fluoride to dental tissues. In regard to this particular posteruptive effect of fluoride on experimental caries, however, the role of fluoride added to the teeth has not been resolved (*17*). Iodoacetate may be effective through a modification of the metabolic activity and products of the oral flora (*18*). Antibiotics likewise may change the spectrum of the oral flora (*19*). It is indicated by these results with penicillin and possibly with iodoacetate that bacterial action within the oral cavity, or possibly in the diet itself (*5*), is involved in this development of smooth surface caries. Whatever the mechanism of their action, these three agents again proved to be significantly effective in preventing experimental dental caries. Fluoride, of course, is also preventive toward the carious process in human beings (*20*). Penicillin has had inconsistent effects on human dental caries (*21, 22*).

The dietary supplement of Osborne-Mendel salts had no significant effect on the percentage of ash, calcium, and phosphorus and on the calcium : phos-

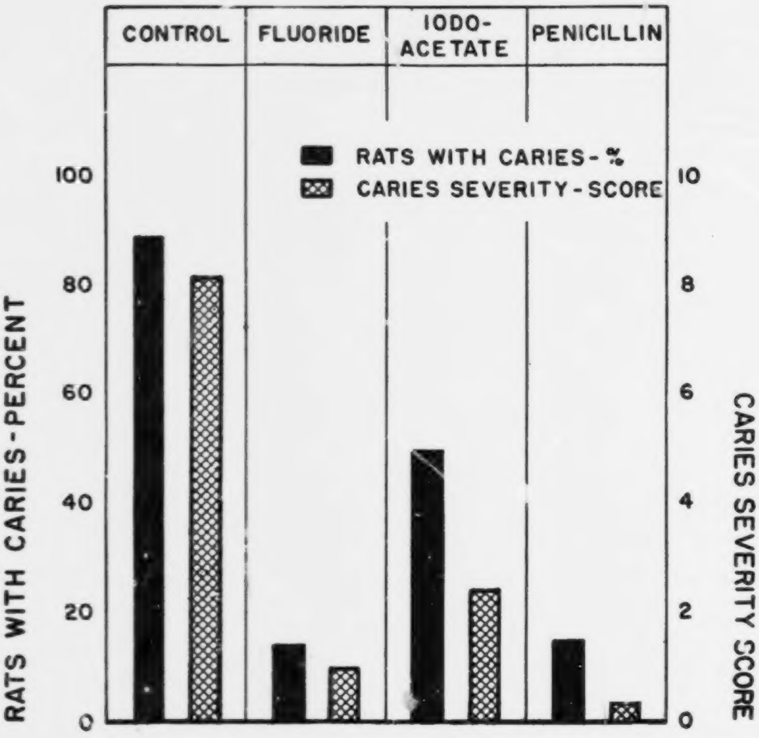

FIGURE 2.—*Effect of fluoride, iodoacetate and penicillin on smooth surface caries produced by diet 636*

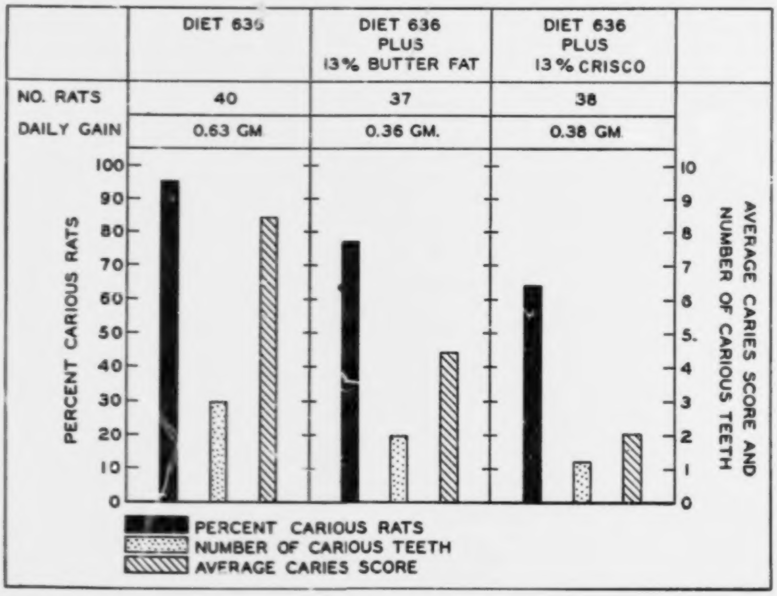

FIGURE 3.—*Effect of butterfat and Crisco on smooth surface caries produced by diet 636*

phorus ratio of the dentin and enamel. It would seem, therefore, that the caries preventive effect of these salts was not due to a change in these mineral components of the dentin and enamel. These additional minerals, it may be emphasized, were ingested after the molar teeth were practically completely calcified. Prepared without the usual sodium fluoride, this salt mixture analyzed 33.8 p.p.m. of fluoride. This diet with added Osborne-Mendel salts analyzed 1.8 p.p.m. of fluoride, an amount too small to have had this caries preventive effect. The validity of this result with Osborne-Mendel salts on smooth surface caries is supported by other unpublished studies by the authors in which Osborne-Mendel salts were preventive toward smooth surface caries usually produced by NIDR diet 586 (*2*).

It may be surmised that the effect of butterfat and Crisco could be due to a change in a physical property of the diets. How this change would prevent caries is purely speculative, but in consideration of the fact that a majority of the smooth surface caries on these studies were observed on lower buccal surfaces, it is realized that physical properties affecting the capacity of the diet to be retained on tooth surfaces could be important. Physical attributes of foods which may affect their retentive property have not escaped previous attention as a factor of cariogenicity (*23*, *8*). Some speculation also has been made that caries prevention by various fats may be related to their degree of saturation. Thus far the evidence has not substantiated this hypothesis (*12*, *13*).

SUMMARY

A posteruptive preventive effect of fluoride, iodoacetate and penicillin on smooth surface caries in white rats was demonstrated. The results compare with previous studies which show that occlusal fissure caries also is significantly prevented by these agents.

A modification of cariogenic diet 636 by replacing equivalent cornstarch with 13 percent Crisco prevented smooth surface caries significantly. Butterfat at this same level in diet 636 did not prevent caries incidence significantly, and the effect on caries severity had questionable significance. Ten percent butterfat replacing an equivalent amount of cornstarch in diet 635, however, prevented smooth surface caries. A supplement of 4 percent Osborne-Mendel salts which replaced 4 percent cornstarch in diet 636 also prevented smooth surface caries significantly. This caries preventive effect occurred practically entirely after molar tooth calcification in the rats was completed.

The objectives of this study were to throw light on the mechanism of the smooth surface caries associated with NIDR diets 635 and 636, as well as to compare this type of caries with occlusal fissure caries. In these regards the response to penicillin, iodoacetate and fluoride suggests similarities in the characteristics of these two types of caries. There is but little information in this study which throws light on fundamental properties of these two diets responsible for their cariogenic effect.

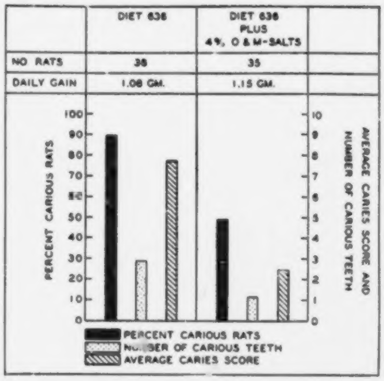

FIGURE 4.—*Effect of 4 percent Osborne-Mendel salts on smooth surface caries produced by diet 636*

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TABLE 2.—*Ash, calcium and phosphorus in teeth of rats given diet 636 compared with those given diet 636 plus 4.0% Osborne-Mendel salts*

Molar	Diet 636				Diet 636 plus Osborne-Mendel salts			
	Ash %	Ca %	P %	Ca:P	Ash %	Ca %	P %	Ca:P
Sound upper—enamel	95.05	38.13	18.23	2.09	93.88	39.58	18.58	2.13
Sound upper—dentin	75.76	38.60	18.35	2.10	75.00	38.21	18.37	2.08
Sound lower—enamel	92.93	39.01	18.78	2.08	95.15	39.66	18.73	2.12
Sound lower—dentin	75.00	37.52	17.98	2.09	74.51	38.12	18.67	2.04
Carious upper—enamel	94.00	38.15	18.17	2.10	*	—	—	—
Carious upper—dentin	75.25	38.00	18.12	2.10	—	—	—	—
Carious lower—enamel	92.93	37.99	17.65	2.15	94.12	39.02	18.68	2.09
Carious lower—dentin	78.79	36.36	17.63	2.06	78.00	38.15	18.17	2.10

*There were no carious upper teeth in this group of rats.

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EFFECT OF SODIUM FLUORIDE ON ORAL LACTOBACILLI ISOLATED FROM THE RAT

By T. Shiota

The inhibition exerted by sodium fluoride on the growth and acid production of Streptococci, Lactobacilli, and other oral microorganisms was observed by Bibby and van Kesteren (*1*). They found a reduction of approximately 50 per cent in acid production by Streptococci and Lactobacilli in media containing 100 p.p.m. NaF. A greater concentration was required, however, to achieve a bactericidal effect. Fitzgerald and Jordan (*2*) found that sodium fluoride and sodium fluorosilicate inhibited the growth of various Lactobacilli, as determined by the broth dilution method. The influence of fluoride on the growth and viability of staphylococci and yeast was demonstrated by Roberts and Rahn (*6*) to depend on the pH of the medium. Fluoride was most efficient in acid media. This was attributed to the existence of fluoride as hydrofluoric acid since the dissociation constant of HF is 7.2×10^{-4}.

This report deals with in vitro changes in the rate and extent of growth, as well as the bactericidal effects of sodium fluoride on various strains of oral Lactobacilli isolated from rats.

MATERIALS AND METHODS

All of the strains of Lactobacilli were obtained from Jordan and Fitzgerald (*3*). The collection consisted of 20 homofermentative Lactobacilli and 20 heterofermentative strains. The homofermentative strains were: pp1, pp3, pp6, pp7, pp8, pp9, 6, 12, 16a, 21, 25, 33, 37, 39, 47, 49, 52, 57, 59, and 84 AT of which strains 84 AT, 16a, and possibly pp7 were *Lactobacillus acidophilus* or variants thereof; the remaining strains are types which have not been previously described in the literature. The heterofermentative strains were variants of *Lactobacillius fermenti*. These were strains 1, 2, 3, 7, 8, 9, 14, 16, 17, 18, 19, 20, 22, 23, 26, 30, 32, 37, 35A, and 38.

Stock cultures were maintained as stabs in Rogosa's SL Medium as modified by Fitzgerald and Jordan (*2*). The medium was adjusted to ph 6.5 and dispensed into tubes containing approximately 0.1 Gm. $CaCO_3$. The tubes were then autoclaved for 10 minutes at 15 pounds. After incubation for 2 days at 37° C., the stock cultures were held at 5° C. and transferred about once a month.

Effects of Sodium Fluoride on Rate and Amount of Growth.—The test medium used for the study of the rate and amount of growth was the tomato juice broth (T.J.) described by Rogosa and Mitchell (*7*). The double strength medium was adjusted to pH 6.8 unless otherwise stated. Solutions of sodium fluoride of varying concentrations, adjusted to the pH of the particular lot of medium being prepared, were added, and the medium was made up to final strength with distilled water, then autoclaved for 5 minutes at 15 pounds. The pH values recorded were taken after sterilization. No effort was made to maintain the pH after inoculation.

The experimental inocula were prepared by inoculating a T.J. broth tube from the stab and transferring twice in the exponential phase of growth. One drop of an 18-hour culture containing approximately 5×10^7 cells were used to inoculate each tube.

The extent of growth at varying fluoride concentrations after varying periods of incubation was measured as optical density at 660 mμ with the Beckman Model B Spectrophotometer, using matched 18 × 150 mm. Pyrex test tubes. The growth response of the organisms to varying fluoride concentrations was plotted as the logarithm of relative optical density (Figs. 1 and 3), which is defined as the logarithm of the ratio of the optical density obtained at any sodium fluoride concentration to that obtained with zero fluoride, times 100 ($\log OD/OD_0 \times 100$) after 48 hours' incubation.

Experiments on Bactericidal Activity of Sodium Fluoride.—In order to determine the bactericidal effects of sodium fluoride on the viability of cells 2 methods were used. In the first, the test was conducted at a given initial pH and the plate count method was used to enumerate surviving cells. In those experiments in which tests were conducted at several pH levels, the broth inoculation pro-

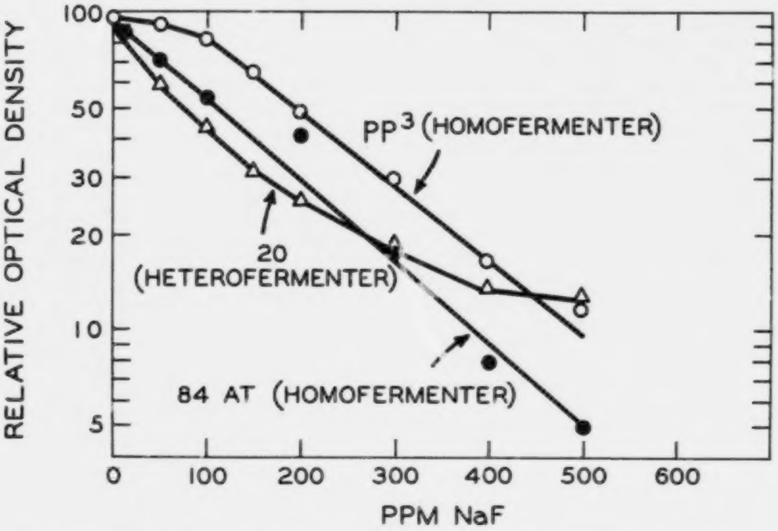

FIGURE 1.—*Effect of NaF on the growth of 3 types of oral Lactobacilli from the rat.*

cedure was employed. In either case the cell suspensions were prepared by taking 10 ml. of a 20-hour-old culture, centrifuging, washing once in sterile distilled water, and standardizing by dilution with sterile water to an optical density of .04. For the plate count method this cell suspension was further diluted 1/10 and 1.0 ml. was added to a sterile tube containing 1 ml. veronal-acetate buffer, pH 6.9 (Michalis (*4*), sodium fluoride at appropriate concentrations (0 to 1,000 p.p.m. NaF) and water to make a final volume of 10 ml. Each mixture was incubated at 37° C. A 1.0 ml. portion from each tube was removed at successive 20-minute intervals over a 2-hour period; each sample was then plated at appropriate dilutions on 10 replicate SL agar plates. These plates were incubated at 37° C. for 72 hours and the colonies counted. The data reported here are derived from the mean counts of 10 replicate plates.

The procedure followed for the second method, in which the effects of sodium fluoride at several pH levels were tested, was as follows: 1.0 ml. of the once-washed cell suspension was added to a sterile tube containing 1.0 ml. of citrate-phosphate buffer (MacIlvaine (*5*) of appropriate pH, NaF solutions of appropriate concentration and pH, and water to make a final volume of 10 ml. The mixture was incubated at room temperature. One-tenth ml. samples were then removed from each tube every half hour for 3 hours and inoculated directly into a tube containing 10 ml. T.J. broth. The latter tubes were then incubated at 37° C. for 48 hours, and any evidence of growth recorded.

RESULTS

Twenty strains each of homofermentative and heterofermentative oral Lactobacilli isolated from rats were tested for growth in the presence of varying sodium fluoride concentrations. Typical fluoride inhibition curves are shown in Fig. 1. Eight homofermentative strains (pp1, pp7, pp9, 16a, 25, 49, and 37) behaved like 84 AT and 12 (pp6, pp8, 6, 12, 21, 33, 29, 47, 52, 57, and 59) like pp3. All of the heterofermentative strains behaved similarily to culture 20. With all of the strains studied, the amount of growth was inversely proportional to fluoride concentration.

The effect on the rate of growth depended upon the strain employed. The growth rate of strain 84 AT, a homofermentative Lactobacillus (Fig. 2), was reduced by 50, 100, 200, and 300 p.p.m. NaF. At sodium fluoride concentrations of 400 or 500 p.p.m. there was an apparent lag, after which the rate of growth was commensurate to that of 300 p.p.m. In contrast, the growth rates of strain pp3, a second homofermentative organism, and strain 20, a heterofermentative organism, were decreased only slightly by as much as 500 p.p.m. sodium fluoride. In some strains, at concentrations of 300 to 500 p.p.m. sodium fluoride, lysis of cells apparently occurred after 9 to 24 hours, depending on the strain, as there was a decrease in the optical density.

Strains 84 AT, pp3, and 20 were further studied to determine the effect of the initial pH of the medium on their sensitivity to fluoride. It was found that for strain 84 AT, fluoride is more effective at lower pH levels, as shown in Fig. 3. The K values for the slopes of the curves of 84 AT calculated by the least squares method according to Snedecor (*9*) indicate the effectiveness of the growth inhibition by sodium fluoride under a given condition. The plot of the K values against the pH values is shown in Fig. 4. It can be seen that good growth occurred over the pH ranges indicated. On the assumption that this inhibition is due to the fluoride ions, the K values were used to calculate the concentration of fluoride (F) necessary to give 50 per cent inhibition for the various pH values which are shown in Table I. Results obtained from cultures pp3 and 20 are also tabulated. The shapes of the inhibition curves for strain pp3 and 20 over the pH range of about 8.0 to 5.0 were similar to those shown in Fig. 1. However at a pH of 4.3 to 4.4, the curves of both pp3 and 20 were similar to 84 AT.

The levels of sodium fluoride neces-

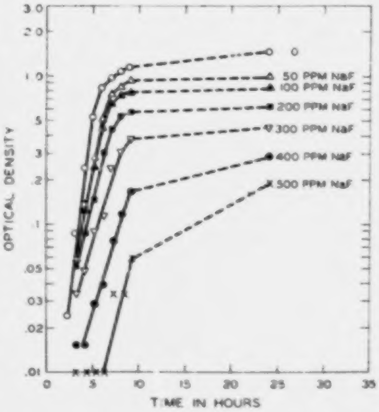

FIGURE 2.—*Effect of sodium fluoride on the growth rate of strain 84 AT.*

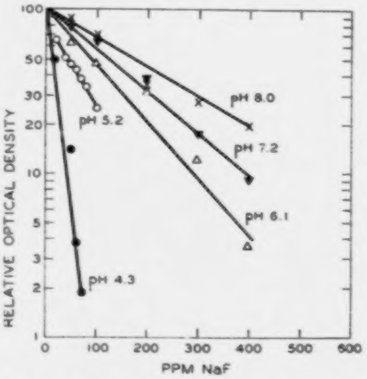

FIGURE 3.—*The effect of pH on the inhibition of growth of strain 84 AT by sodium fluoride.*

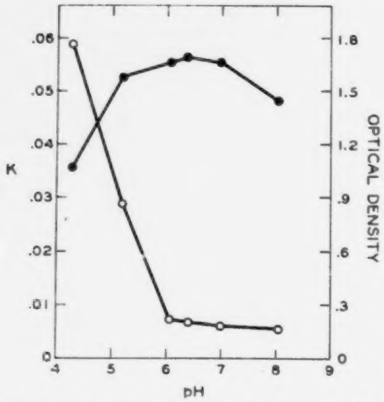

Figure 4.—*The effect of pH on the growth of strain 84 AT and the effectiveness of the growth inhibition by sodium fluoride.* ○——○ *K,* ●——● *=optical density of controls.*

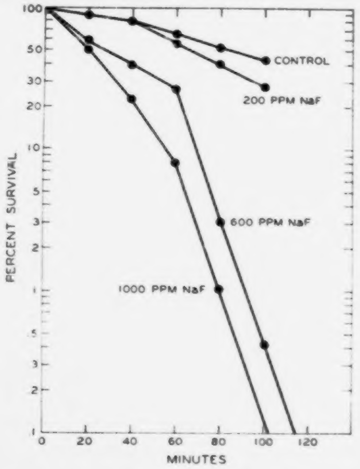

Figure 5.—*The bactericidal effect of sodium fluoride on cell suspensions of strain at 84 AT.*

sary to achieve a bactericidal effect on strain 84 AT are shown in Fig. 5. Nearly 2 hours were required to achieve a 99.9 per cent kill with bacterial suspensions of this strain exposed to 600 p.p.m. NaF at pH 6.9. Experiments were then performed with this strain to test the influence of pH values on the bactericidal effect of sodium fluoride. Results are shown in Table II. It can be seen that in the range of pH 4.0 to 6.0, the exposure of cells to 200 p.p.m. NaF for 2 hours was sufficient to prevent the growth of organisms in subcultures from aliquots tested; whereas at pH 7.0 or above, growth occurred from subcultures exposed to 1,000 p.p.m. NaF for 3 hours. In some experiments at pH 4.0, after 3 hours with no sodium fluoride added, subcultures failed to grow.

Table I.—*Concentration of sodium fluoride necessary to give a 50 per cent inhibition*

84 AT			pp3		20	
pH	−K	p.p.m. F.*	pH	p.p.m. F.**	pH	p.p.m. F**
4.3	.0575	5.4	4.4	33	4.3	14
5.2	.0289	11.2	5.0	81	5.6	27
6.1	.0078	39.8	5.7	81	5.9	36
6.4	.0070	44.3	6.0	81	6.7	41
7.2	.0065	46.8	6.3	81	7.3	45
8.0	.0053	58.5	7.0	95	7.8	68

*$F_{50}\ \% = (.45) - \frac{2.3}{K} \log \frac{1}{2}$

= p.p.m. fluoride (F) to give 50 per cent inhibition of growth.

**Estimated from graphs.

DISCUSSION

The various effects of fluoride on the growth of homofermentative and heterofermentative Lactobacilli are indicative of the distinct biochemical composition of these 2 groups. Moreover, within the homofermentative Lactobacillus group, there might be further distinctions reflected by their sensitivity toward fluoride, as seen in Fig. 1. At the lower fluoride levels, the type represented by pp3 is more resistant toward fluoride than that of 84 AT. However, at higher levels, the rate of inhibition parallels that of strain 84 AT.

The similar rate of growth of strain 84 AT at 500 p.p.m. sodium fluoride as compared to 300 p.p.m. is difficult to explain in view of the decrease in the total growth. The apparent lag period, which occurred at the higher sodium fluoride concentrations, may not be a true lag since the initial number of cells introduced as inoculum may have been reduced by the bactericidal action of sodium fluoride. This possible reduction was not tested in the growth experiments.

The results of both the growth experiments and the bactericidal experiments are in agreement in indicating that the antibacterial activity of sodium fluoride for the oral Lactobacilli isolated from the rat is dependent on pH. The inhibitory effects of sodium fluoride are most pronounced at the lower pH levels presumably due to the higher concentrations of the undissociated HF in this range.

SUMMARY

When varying concentrations of sodium fluoride were used to inhibit the growth of 20 strains each of homofermentative and heterofermentative oral Lactobacilli isolated from rats, 3 groups were distinguishable, as shown by the characteristic shapes of their growth inhibition curves. The homofermentative Lactobacilli were divided into 2 groups: in one, typified by strain 84 AT, a monomolecular reaction was observed and in a second, illustrated by strain pp3, a resistance to sodium fluoride through 50

Table II.—*The influence of pH on the bactericidal effect of sodium fluoride on cell suspensions of strain 84 AT*

pH	NaF p.p.m.	EXPOSURE TIME						
		0	½	1	1½	2	2½	3
4	0	+	+	+	+	+	+	−
	20	+	+	+	+	+	+	−
	200	+	+	+	−	−	−	−
	600	+	+	+	+	−	−	−
	1000	+	+	+	+	−	−	−
6	0	+	+	+	+	+	+	+
	20	+	+	+	+	−	+	+
	200	+	+	+	−	−	−	−
	600	+	+	+	−	−	−	−
	1000	+	+	+	+	−	−	−
6.5	0	+	+	+	+	+	+	+
	20	+	+	+	+	−	−	−
	200	+	+	+	−	+	−	−
	600	+	+	+	+	−	−	−
	1000	+	+	+	+	+	−	−
7	0	+	+	+	+	+	+	+
	20	+	+	+	+	+	+	+
	200	+	+	+	+	+	+	+
	600	+	+	+	+	+	+	+
	1000	+	+	+	+	+	+	+
8.2	0	+	+	+	+	+	+	+
	20	+	+	+	+	+	+	+
	200	+	+	+	+	+	+	+
	600	+	+	+	+	+	+	+
	1000	+	+	+	+	+	+	+

p.p.m. was noted. The third group, represented by strain 20, was composed of heterofermentative Lactobacilli. The growth rate of strain 84 AT was reduced by 50, 100, 200, and 300 p.p.m. NaF. At concentrations of 400 or 500 p.p.m., there was an apparent lag after which the rate of growth was approximately the same as that at 300 p.p.m. With strains pp3 and 20, rates of growth were decreased only slightly through 500 p.p.m. NaF. The effect of pH on the inhibition of growth by sodium fluoride indicated that as the pH of the growth medium was lowered from pH 8 through 4, the inhibitory action of sodium fluoride against strains 84 AT, pp3, and 20 was increased. In bactericidal experiments 99.9 per cent of cells of strain 84 AT were killed when they were exposed to 600 p.p.m. NaF for 2 hours at pH 6.9. The bactericidal effect of sodium fluoride was increased as the pH of the testing mixture was decreased from pH 8 to 4.

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Fluoride Metabolism and Retention in Calcified Tissues

The retention of fluoride in calcified tissues is a dominating characteristic of fluoride metabolism. Because of the unusual extent to which fluoride may become cumulative, a great deal of attention has been directed to problems concerning its absorption retention and elimination from the body. Cumulative effects of fluoride may be predicted by the quantity of the ingested fluoride which the body regularly retains or eliminates. Bone abnormalities and systemic illness occur when the tolerance levels of fluoride are exceeded.

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THE EFFECT OF FLUORINE ON THE CALCIUM METABOLISM OF ALBINO RATS AND THE COMPOSITION OF THE BONES

By F. J. McClure and H. H. Mitchell

INTRODUCTION

It has been shown by the experiments of Forbes and coworkers (*8*) and Bethke, Kick, Edgington, and Wilder (*2*) with swine, by Tolle and Maynard (*32*) with rats, Reed and Huffman (*22*) with dairy cattle, and Buckner, Martin, and Peter (*4*) with poultry, that phosphorite minerals used as calcium supplements in animal nutrition may exert harmful effects. These effects may be of a definitely toxic type, such as impaired appetite, disturbances of the general health of the organism, and more specifically a faulty bone and tooth development. Reported analyses of commercial grades of rock phosphate show the presence of from 3 to 4 percent of fluorine (*11*). In view of the known toxic properties of this element, it is not surprising that it is generally believed, though not definitely proved, that fluorine is responsible for the unfavorable results obtained with rock phosphate in animal feeding. In at least two instances effects comparable in nature to those observed by feeding raw rock phosphate have been related to fluorine additions to the ration. The work of Bethke and coworkers (*2*) at the Ohio Experiment Station shows that the fluorine content of the bones may be increased by the feeding of rock phosphate as well as by sodium fluoride. Tolle and Maynard (*32*) demonstrated that the feeding of rock phosphate produced an effect on the teeth of rats known to be characteristic of fluorine. It was the purpose of this investigation, therefore, to determine the effects of fluorine on animals, particularly on the metabolism of calcium and on the composition of the bones, and thus to throw some light upon the effects to be expected from rock phosphate when used as a mineral supplement to live stock rations, by reason of its fluorine content.

EFFECTS OF FLUORINE REPORTED IN THE LITERATURE

Numerous cases are on record in which fluorine in one form or another, taken *per os* or by injection in comparatively small amounts, has proved to be extremely toxic and very often fatal. Baldwin (*1*) and McNally (*19*) have listed several cases of fluorine poisoning in human beings. Tappeiner (*33*), Schulz (*24*), Pitotti (*21*), Wieland and Kurtzahn (*35*) Brandl and Tappeiner (*3*), Schwyzer (*26, 27*), and others have shown that small dosages of fluorine in various forms and amounts may retard the growth and otherwise impair the normal development and functioning of the organism.

Sollmann, Schettler, and Wetzel (*28*) have studied chronic intoxications in albino rats, including in their studies sodium fluoride and phosphate rock, being primarily concerned with the probable toxicity of fluorine. Their method consisted in adding a pure form of sodium fluoride to the ration and observing its effect on growth and food consumption. Generally their results show a parallelism between the food intake and the growth of the rats. In three instances, however, the food consumption was normal or above, whereas the rate of growth was below normal. The amount of fluorine in the rations in these cases varied from 0.018 to 0.0452 percent. These results may be regarded as indicating an effect of fluorine at these levels of intake on the rate of growth of rats. An amount of fluorine equivalent to 0.103 percent of the ration proved fatal to seven rats within 11 weeks. Six rats received a ration containing 0.045 percent of fluorine along with a normal ration, both fed *ad libitum*. Three of these rats died by the end of the 5th week. The results show that all the fatalities occurred on the higher level of fluorine feeding, so that it may be concluded that fluorine was the causal factor.

Schulz and Lamb (*25*) added sodium fluoride to a basal ration which had proved adequate for normal growth and reproduction of rats, in amounts representing 0.0226, 0.0452, 0.0678, and 0.1130 percent fluorine. The rats receiving 0.0226 percent fluorine remained normal, those receiving 0.0452 percent "did not grow as well or as rapidly as the first group." Out of six litters of young in this lot, four were reared, but these grew "at a very slow rate." Rats receiving 0.0678 percent fluorine grew at about the same rate as the second group. Out of four litters produced, two litters lived. Of seven rats fed 0.1130 percent fluorine in the ration, all died within 8 to 14 weeks and none of the lot reached 100 gm. in weight. It is evident that the higher amount of fluorine is definitely toxic, but it is difficult to interpret the effect of the lower amounts in the absence of data concerning the food intake and the rate of growth of the individual rats. Regarding the effect of fluorine on reproduction the authors state that "It appears than an unfavorable effect on reproduction begins at a level of about 0.0250 percent sodium fluoride [0.0102 percent fluorine] in the ration."

In an attempt to duplicate the toxic effects obtained by feeding raw rock phosphate to dairy cattle, Taylor (*31*) at the Michigan Agricultural Experiment Station has fed several dairy cows varying amounts of fluorine in the form of calcium fluosilicate. The effects noted in this case were a loss of appetite, and a rapid decline in weight. That the teeth of the animals were being affected was indicated by the tendency to lap water. The teeth at this time were observed to be rather narrow and the animal was slightly shear-mouthed. The grinding surfaces of the molars were eventually worn down and the nerves exposed.

The peculiar effect of fluorine on the quality of the teeth of rats was first observed by McCollum, Simmonds, Becker, and Bunting (*17*). Sodium fluoride to the extent of 0.05 percent (0.0226 percent fluorine) was included in an otherwise normal diet for rats. The abnormal growth of the incisor teeth observed appears to be due to changes in structure and hardness of the teeth, which results in a lack of the natural wear which the incisors of the rat exert on each other in occlusion under normal conditions.

The ability of ingested fluorine to modify the structure of the teeth of the rat and dairy cow may be related to its deposition in the teeth to abnormally high levels. That fluorine is a normal constituent of body tissues has been shown by Gautier (*9*), Zdarek (*37*), Wrampelmeyer (*36*), Harms (10), Jodlbauer (*12*), Sonntag (*29*), and others. The fluorine content of teeth, judged by the reported analyses, amounts to about 0.2 to 0.3 percent and is the highest of any body tissue, the average for normal bone would equal about 0.03 to 0.10 percent. Sonntag (*29*) found that whereas normal teeth and bones of dogs contained not above 0.3 percent of fluorine, the bones of dogs fed sodium fluoride contain 1.73 percent on the moisture- and fat-free basis and the teeth 1.29 percent on the dry basis. Trebitsch

(*34*) as well as Brandl and Tappeiner (*3*), have reported the deposition of abnormal amounts of fluorine in various body tissues by continuous small additions of fluorine to the ration. As previously mentioned Bethke and coworkers (*2*) have furnished evidence of the ability of fluorine, either as sodium fluoride or in the form in which it occurs in rock phosphate, to increase uniformly the amount of fluorine contained in the bones. Pigs fed limestone and steamed bone meal only, had 0.0409 percent and 0.0231 percent fluorine, respectively, in the dry fat-free bone. The lots of pigs receiving 30, 60, and 100 gm. of sodium fluoride and 2 pounds of limestone per 100 pounds of basal ration had respectively 0.528, 0.793, and 1.108 percent fluorine in the dry fat-free bone.

Attempts to demonstrate an effect of fluorine on the composition of various tissues other than an increase in the fluorine content have been generally unsuccessful. Bethke and associates (*2*) analyzed the bones of pigs fed sodium fluoride, as well as of those fed rock phosphate, for ash and for the calcium, magnesium, and phosphorus content of the ash, but found no significant differences from the normal bone. Schwyzer (*26*) observed a slight decrease in the specific gravity of the bones of fluorine-fed rabbits. The reported results of the calcium and chlorine analyses of the bones and the blood of animals fed fluorine by Schwyzer (*26, 27*) show no consistent variations from the normal. However, the blood of rabbits, guinea pigs, dogs, and pigeons fed fluorine according to Schwyzer shows a very high degree of coagulability and an abnormal ratio between the uninuclear and multinuclear leucocytes.

A well demonstrated property of fluorides, is their inhibiting effect on enzyme action. Loevenhart and Peirce (*14*) studied the effect of sodium fluoride on the action of lipase. Measuring the decrease in the amount of acid produced by the action of the enzyme present in a pancreatic extract on olive oil and ethyl butyrate, these workers found sodium fluoride in dilutions of 1:5000 to have an inhibiting effect of 3 percent for olive oil and 70 percent for ethyl butyrate. With clear liver extracts as sources of lipase, dilutions of NaF of 1:5,000,000 inhibited the action of lipase on ethyl acetate 50 percent. Loevenhart and Peirce tested the effect of a large variety of other salts and found this marked inhibiting effect to be characteristic of fluorides. Lang and Lang (*13*) have shown that the decomposition of starch by pancreatic juice is checked by the action of sodium fluoride. It appears that fluorine does not affect the action of pancreatic maltase in the hydrolysis of maltose to glucose but does have an inhibitory effect on the conversion of starch to maltose. Clifford (*5, 6*) has studied extensively the effect of halogen salts in general on enzyme digestion, using as her method of study, in the case of pepsin, the effect of the various salts on the time required for milk to begin to clot. The reaction was inhibited by concentrations of sodium fluoride greater than 0.0144 mol per liter (*6*).

It is probable that the effect of fluorides on the reactions of muscle and nerve cells may be characteristic of calcium precipitants in general. It is known that muscles taken out of a solution of sodium fluoride, sodium carbonate, sodium acid phosphate, sodium oxalate, citrate, or tartrate and let stand in the air contract powerfully. The nerve placed in the same solutions begins to twitch and goes into tetanus. Robertson and Burnett (*23*) believe that the above effects are due to a disturbance of the balance of calcium. The effect of the fluorine ion on the chemical changes occurring in the contracting muscle has been investigated by Embden and his school. One rather remarkable effect of fluorine is the preventing of the hydrolysis of the hexosephosphate complex in muscle, a compound formed in the intermediary metabolism of glycogen.

It cannot be said that there is available experimental evidence aside from the work of Bethke and associates (*2*) at the Ohio Agricultural Experiment Station as to the effect (if any) of fluorine compounds on bone development and calcium metabolism. The most generally known as well as perhaps the most definite effect of the intake of a small amount of fluorine is that produced on the quality of the teeth of rats. Because of similarities in metabolism and composition between bone and tooth, an effect of fluorine on bone composition may be at once surmised. The effects of fluorine on enzyme action are of interest, since they might become a factor in the digestion and utilization of rations where fluorine in abnormal amounts is present. How serious the addition of small amounts of fluorine may become as regards the effect on the growth and general health of the organism cannot be definitely stated from the experiments reported in the literature, though the results of practical feeding trials indicate that serious effects may follow, as was the case in the feeding trials with dairy cattle. Small amounts of fluorine have no doubt influenced the food intake and probably the growth of rats fed by Sollmann (*28*). The results of Schulz and Lamb (*25*) also indicate that fluorine may retard growth and hinder normal reproduction in rats.

PLAN OF EXPERIMENT

It has not been the purpose of this experiment to obtain an extreme toxic

TABLE I.—*Effect of fluorine as sodium fluoride and calcium fluoride on growth of white rats*

Rat No.	F present	Ca in ration	Days on experiment	Average daily food intake	Average daily gain in weight	Rat No.	F present	Ca in ration	Days on experiment	Average daily food intake	Average daily gain in weight
Sodium fluoride fed						Calcium fluoride fed					
	percent	*percent*		*gm.*	*gm.*		*percent*	*percent*		*gm.*	*gm.*
1	0.0106	0.154	90	8.22	1.63	19	0.0313	0.315	84	8.54	1.83
2	0.0004	0.148	90	8.13	1.28	20	0.0004	0.294	84	8.62	1.96
3	0.0106	0.154	95	7.98	1.57	21	0.0313	0.315	84	7.49	1.48
4	0.0004	0.148	95	8.17	1.71	22	0.0004	0.294	84	7.49	1.78
5	0.0106	0.154	95	8.16	1.56	23	0.0313	0.315	84	7.97	1.90
6	0.0004	0.148	95	8.14	1.56	24	0.0004	0.294	84	8.05	1.82
7	0.0313	0.341	93	8.88	1.47	25	0.0313	0.315	84	7.78	1.80
8	0.0004	0.341	93	8.81	1.67	26	0.0004	0.294	84	7.83	1.86
9	0.0313	0.341	83	7.78	1.00	27	0.0623	0.615	80	8.45	1.94
10	0.0004	0.341	83	7.69	1.14	28	0.0004	0.569	80	8.48	1.94
11	0.0313	0.341	90	7.61	1.12	29	0.0623	0.615	80	7.34	1.56
12	0.0004	0.341	90	7.55	1.08	30	0.0004	0.569	80	7.29	1.79
13	0.0623	0.596	86	6.65	0.88	31	0.0623	0.615	79	7.55	1.96
14	0.0004	0.634	86	6.64	0.91	32	0.0004	0.569	79	7.52	1.95
15	0.0623	0.596	81	6.29	0.80	33	0.0623	0.615	78	6.76	1.24
16	0.0004	0.634	81	6.36	0.91	34	0.0004	0.569	78	6.68	1.67
17	0.0623	0.596	80	6.10	0.85						
18	0.0004	0.634	80	6.37	0.99						

TABLE II.—*Effect of fluorine as sodium fluoride on the calcium balance of white rats*[1]

Period No.	Rat No. and treatment	Ca intake	Ca in urine	Ca in feces	Ca balance	Ca retained
		0.0106 percent fluorine				
		mg.	*mg.*	*mg.*	*mg.*	*percent*
1	1. Fluorine	125.6	5.0	37.9	82.7	65.8
	2. Control	117.4	3.7	66.1	47.6	40.5
2	1. Fluorine	130.7	2.5	28.9	99.3	75.9
	2. Control	126.1	2.5	25.9	100.2	79.4
1	3. Fluorine	104.4	5.0	44.0	55.4	53.0
	4. Control	98.2	3.7	51.7	42.8	43.5
2	3. Fluorine	134.5	3.7	20.7	110.1	81.8
	4. Control	132.1	2.5	29.2	100.4	76.0
1	5. Fluorine	115.8	5.0	89.0	21.8	18.8
	6. Control	106.5	5.0	60.1	41.3	38.7
2	5. Fluorine	141.0	3.7	60.2	78.3	55.5
	6. Control	134.5	3.7	48.4	82.4	61.2
		0.0313 percent fluorine				
1	7. Fluorine	306.1	23.7	82.4	200.0	65.3
	8. Control	314.2	28.7	93.4	192.1	61.1
2	7. Fluorine	323.7	18.7	160.0	145.0	44.7
	8. Control	338.1	15.0	168.1	155.0	45.8
1	9. Fluorine	235.6	16.2	87.7	131.7	58.3
	10. Control	238.3	15.0	73.4	149.9	62.9
2	9. Fluorine	306.7	17.5	161.3	126.9	41.3
	10. Control	295.6	22.5	128.3	138.8	46.9
1	11. Fluorine	247.3	11.2	102.3	133.8	54.1
	12. Control	256.9	8.7	68.9	179.3	69.7
2	11. Fluorine	279.8	11.2	115.0	153.6	54.8
	12. Control	287.4	12.6	152.2	122.7	42.6
		0.0623 percent fluorine				
1	13. Fluorine	357.5	18.7	180.3	158.5	44.3
	14. Control	402.5	17.5	150.1	234.9	58.3
2	13. Fluorine	405.6	25.2	275.0	105.4	25.9
	14. Control	408.7	21.5	240.2	147.0	35.9
1	15. Fluorine	316.7	18.7	150.3	147.7	46.6
	16. Control	357.4	13.7	143.1	200.6	56.1
2	15. Fluorine	378.6	10.0	294.7	83.9	22.1
	16. Control	388.7	18.7	148.3	221.7	57.0
1	17. Fluorine	326.8	53.5	163.7	109.6	33.5
	18. Control	362.5	12.8	206.3	143.4	39.5
2	17. Fluorine	392.4	25.2	210.2	157.0	40.0
	18. Control	393.1	17.5	289.6	96.0	24.4

[1] The figures are totals for 10 day collection periods.

effect of fluorine, but rather to keep the amount of added fluorine at a low concentration, comparable with concentrations that may very well result in the practical feeding of raw rock phosphate as a mineral supplement to live stock, particularly in swine rations. Young growing rats were used as the experimental animals and were fed sodium fluoride in the ration in amounts equivalent to approximately 0.01, 0.03, and 0.06 percent of fluorine. Two levels of calcium fluoride providing about 0.03 percent and 0.06 percent of fluorine were also fed to growing rats. Since the most definitely characterized deleterious effect of the feeding of raw rock phosphate to animals relates to the structure and composition of bone and teeth, the investigation was planned to study primarily the growth, bone and tooth development, and the utilization of calcium of the experimental animals.

Male rats weighing from 60 to 80 gm. were paired so that the pair mates were as nearly the same weight as possible. Each rat was placed in a separate cage with wire mesh bottom and provided with heavy glass cups as containers for water and feed. The paired feeding method was followed throughout the experiment, the purpose being to give to each rat of a pair practically the same amount of feed daily as its pair mate received, the rat voluntarily consuming the least setting the pace for its pair mate. The rats of each pair received the same ration with the exception of fluorine, contained in the ration of one rat of each pair either as sodium fluoride or calcium fluoride. The degree to which the food consumption was kept the same for each pair of rats is indicated in tables I and II. In six pairs, the total food consumption was the same for each rat of the pair, in nine pairs it differed by 1 to 2 percent, in one pair it differed by 2.5 percent, and in one pair (Rats 17 and 18) it differed by over 4 per cent. The rats were weighed every 10 days.

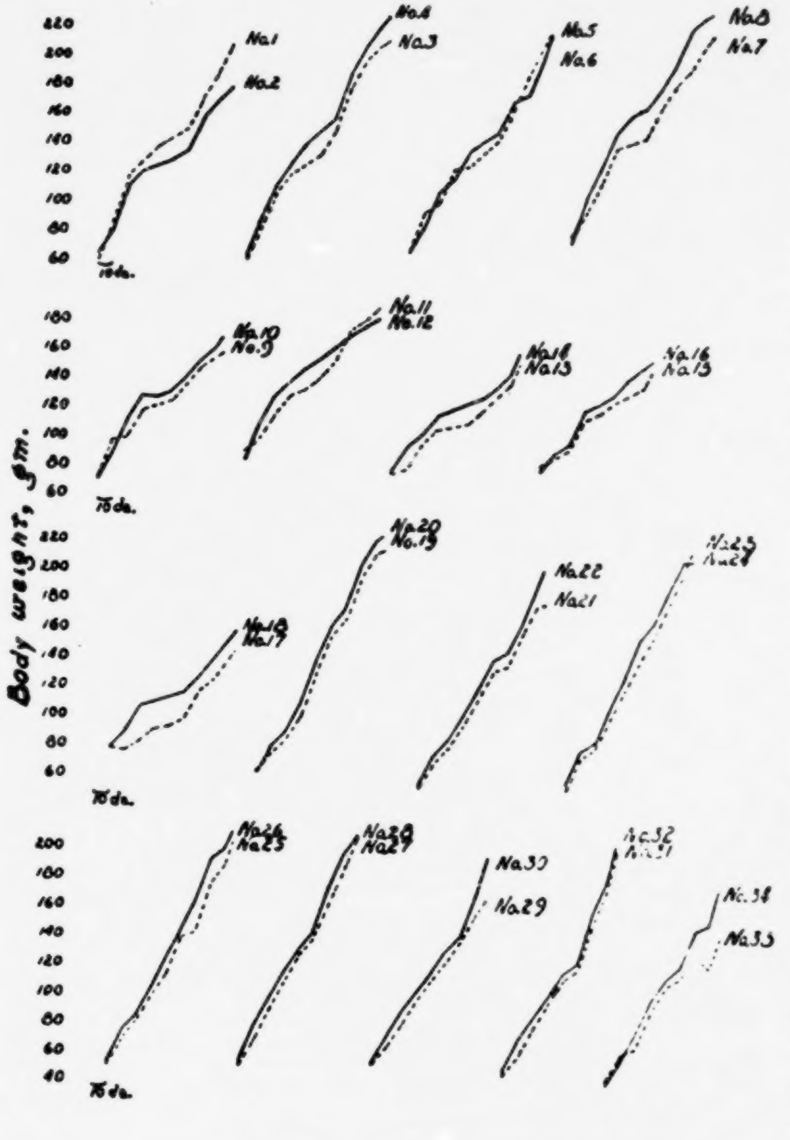
The basal ration fed was as follows: sucrose 10, Cellu Flour 4, casein 15, butter fat 10, starch 56, sodium chloride 1, Osborne and Mendel's Ca-free salt mixture (*20*) 4 per cent. The starch used was partially dextrinized by pouring boiling hot water over it, with continual stirring. It was then dried rapidly in a hot air bath and ground fine. Merck's casein prepared according to Hammarsten was fed. It contained 0.035 percent calcium. The rats were fed, in addition to the basal ration, 2 drops of cod liver oil and 0.2 gm. of Northwestern Yeast Company's yeast powder daily.

All the rats were carried through two 10 day balance periods. The first balance periods occurred during the first 6 weeks of the experiment, the second periods during the latter 4 weeks. During these periods the rats were placed in metabolism dishes of the type described by Mitchell (*16*) for use in determining biological values of proteins. The food intake was controlled and was accurately weighed as in the other feeding periods. Calcium was determined in the rations, feces, and urine by the method of McCrudden (*18*).

EFFECT OF SODIUM FLUORIDE AND CALCIUM FLUORIDE ON GROWTH AND FOOD INTAKE OF RATS

From the data in table I and chart 1, it is apparent that the three rats receiving 0.0106 percent fluorine in their ration showed no consistent differences in rate of gain from their pair mates not receiving fluorine. One pair gained at exactly the same average rate, while in another pair the fluorine-fed rat gained more rapidly, and in the third pair the rat not receiving fluorine gained the more. Of the three pairs of rats used in testing the ration containing 0.0313 percent fluorine, the control rat made the greater gain in two cases, amounting to 0.20 gm. and 0.14 gm. daily. In the

CHART 1.—*Curves showing rate of growth of pairs of rats. The treatment of each rat of a pair was identical except that fluorine was present in the ration of the odd numbered rat of each pair*



other pair the daily rate of gain averaged 0.04 gm. more for the fluorine-fed rat. All of the control rats of the pairs receiving 0.0623 percent fluorine as sodium fluoride made slightly greater daily gains than did their pair mates. Of the six pairs of rats involved in the two higher levels of fluorine feeding, in five of the pairs the control rat showed a slightly greater average rate of gain than did its pair mate, the fluorine-fed rat. On the other hand, it must be admitted that the greater gain of the control rat in the last pair may have been the result largely of its greater intake of food, which averaged 104.4 per cent of that of its pair mate.

The average daily intake of the rats receiving 0.0623 percent fluorine and of their controls was 6.40 gm., while for the pairs in the other two groups, the averages were 8.05 and 8.13 gm., respectively. It seems evident that the highest level of fluorine in the ration caused a lowering of the amount of food consumed and of the rate of growth induced.

Eight pairs of rats were compared on two levels of fluorine as calcium fluoride, namely 0.0313 percent and 0.0623 percent. The effects on growth and food intake appear in table I and in chart 1. In three pairs of rats used in testing rations containing 0.0313 percent fluorine, the rat not receiving fluorine made a slightly greater average daily gain; i.e., 0.13, 0.30, and 0.06 gm. In the fourth pair the fluorine-fed rat made gains averaging 0.08 gm. greater per day than its pair mate control. However, in the latter pair the control rat exceeded the fluorine-fed rat in body weight throughout the experiment except for the final weight. This relation between control and test rats held throughout the experiment for the other three pairs. The growth curves given in Chart 1, more clearly than the data of table I, indicate an inhibiting effect of the fluorine ration on growth.

Also at a level of 0.0623 percent of the daily ration, fluorine as calcium fluoride appears to have retarded growth. In average gain the control rat exceeded the fluorine-fed rat considerably (0.23 and 0.43 gm. daily) in two pairs and made practically the same gain in the other two. But on reference to chart 1, it is seen that in all pairs at this level of calcium fluoride, except for the final weights on rats 27 and 28, the control rat maintained throughout the experiment a greater body weight than its pair mate, whether or not it started with such an advantage. Furthermore in the last pair of rats the difference between the control rat and its pair mate widened markedly and progressively in the last half of the feeding period. Thus, with calcium fluoride as with sodium fluoride the indications are definite that the fluorine inhibits growth, entirely aside from its effect on food consumption.

The average daily food intake of rats 19 to 26 inclusive was 7.97 gm., and that of rats 27 to 35 inclusive, 7.51 gm. The higher levels of fluorine intake as CaF_2 have thus had little if any effect in lowering the food consumption.

It may be shown by a mathematical analysis of the growth data, more definitely than is possible by mere inspection, that sodium and calcium fluoride inhibited the growth of the rats fed 0.0313 per cent fluorine or more. The average of the 14 differences in average daily gain between pair mates equals +0.119 ±0.025 gm., the positive sign indicating the greater average gain of the control rats. The standard deviation of this average difference is 0.0365, which will go into the average difference 3.26 times. From the values of the normal probability integral (7), it may be computed that an average difference of this size or larger would occur by chance only once in 893 trials. Hence this outcome could hardly have been the result of chance, but must have been a fluorine effect.

In summarizing the effect of fluorides on growth and food intake it may be said with respect to either calcium fluoride or sodium fluoride that at levels of 0.0313 percent and 0.0623 percent in the ration, fluorine inhibits growth, entirely aside from any effect on food consumption. The higher level of sodium fluoride appears to have lowered the food consumption of the rats also, but the corresponding level of calcium fluoride had no apparent influence on food consumption.

Effect of Sodium Fluoride and Calcium Fluoride on Calcium Balance of Rats

A complete record of the calcium intake and output of the rats in the sodium fluoride balance experiment, determined at two different times during the course of the experiment, appears in table II. A similar record of the calcium fluoride balance experiment will be found in table III. In table IV are recorded the percentages of the calcium intake retained for each balance period, and the differences in this respect between the rats of each pair.

Since the calcium intake of pair mates was not the same, due to the fact that there were slight differences in the percentages of calcium in the rations, or to differences in the total food intake for the balance period, the percentage retention of calcium seems more significant than the absolute retention. It is evident that there were no significant differences in the absolute retention of calcium, nor in the percentage retention, between the rats receiving 0.0106 percent and 0.0313 percent fluorine as sodium fluoride, and their pair mates. The average difference (control rat minus fluorine-fed rat) is −2.1 percent for the pairs concerned with the 0.0106 percent level of fluorine, and +1.6 percent for pairs concerned with the 0.0313 percent level of fluorine. The average percentage of the calcium intake retained by the former pairs is slightly greater than that of any of the other groups of rats. The absolute amounts of calcium retained by these rats are, however, smaller than those retained by the pairs testing the higher levels of fluorine intake, no doubt due to the smaller amounts of calcium consumed by them. However, as far as the composition of the bones indicates (see table V), these smaller amounts in the ration were adequate to meet the requirements of the rats.

TABLE III.—*Effect of fluorine as calcium fluoride on the calcium balance of white rats*[1]

Period No.	Rat No. and treatment	Ca intake	Ca in urine	Ca in feces	Ca balance	Ca retained
	0.0313 percent fluorine					
		mg.	*mg.*	*mg.*	*mg.*	*percent*
1	19. Fluorine	282.9	26.4	108.7	147.8	52.2
	20. Control	267.8	22.5	76.8	168.5	62.9
2	19. Fluorine	364.7	47.9	141.6	175.1	48.0
	20. Control	319.3	50.0	146.2	123.2	38.6
1	21. Fluorine	275.1	28.5	56.9	189.7	68.9
	22. Control	260.5	25.8	62.4	172.2	66.1
2	21. Fluorine	333.3	55.4	110.0	167.9	50.4
	22. Control	300.6	52.0	97.9	150.7	50.1
1	23. Fluorine	256.7	19.0	65.6	172.1	67.0
	24. Control	236.8	28.4	55.7	152.7	64.5
2	23. Fluorine	361.1	48.7	124.2	188.1	52.1
	24. Control	324.9	49.9	114.1	160.9	49.5
1	25. Fluorine	251.4	30.0	56.9	164.5	65.4
	26. Control	236.2	28.9	64.0	143.4	60.9
2	25. Fluorine	331.4	43.1	179.0	109.3	33.0
	26. Control	301.2	49.5	142.5	109.2	36.2
	0.0623 percent fluorine					
1	27. Fluorine	512.3	39.5	315.4	197.0	38.4
	28. Control	491.1	30.0	331.0	130.1	26.5
2	27. Fluorine	690.0	52.5	495.3	142.2	20.6
	28. Control	645.7	50.4	423.0	172.3	26.7
1	29. Fluorine	519.1	34.1	361.5	125.7	24.2
	30. Control	456.8	41.4	261.0	156.7	34.3
2	29. Fluorine	548.5	44.3	402.1	102.2	18.6
	30. Control	515.9	48.8	322.0	145.0	28.1
1	31. Fluorine	457.2	24.1	299.1	134.9	29.5
	32. Control	414.7	23.0	261.1	130.6	32.3
2	31. Fluorine	673.9	64.0	408.6	131.3	19.5
	32. Control	627.0	51.3	329.9	245.8	39.2
1	33. Fluorine	407.6	30.7	283.4	93.5	22.9
	34. Control	359.3	34.2	182.2	142.9	39.8
2	33. Fluorine	463.3	47.4	315.1	100.7	21.7
	34. Control	477.3	41.0	319.4	116.8	24.5

[1] The figures are totals for 10 day collection periods.

With the exception of rats 17 and 18, period 2, the fluorine-fed rats receiving a ration containing 0.0623 percent fluorine in the form of NaF showed smaller percentage retentions of calcium than did their pair mates. The average difference is 9.8 percent in favor of the rat not receiving fluorine. In all but one period out of six, a smaller absolute calcium balance was also obtained for the fluorine-fed rat. The differences in the amount of calcium retained by the fluorine-fed rat below that of its pair mate varies from 33.8 to 137.8 mg. That this level of fluorine intake has resulted in a depression of the retention of calcium seems apparent.

An amount of fluorine in the form of calcium fluoride equivalent to 0.0313 percent of the ration had no apparent detrimental effect on the calcium balance, as shown in tables III and IV. In this series of experiments, the amount of calcium retained is less for the control rat in all but one instance than it is for the pair mate receiving fluorine. Owing to a slightly smaller average calcium content of the rations fed the control rats, the calcium intake of these rats was less than that of their pair mates receiving fluorine. Expressed as a percentage of the calcium intake, the differences in calcium retention between the individuals of a pair are small. All but two of the differences favor the fluorine-fed rat, but the average difference is only −1.0 percent.

In all but one balance period the rats receiving 0.0623 percent fluorine as calcium fluoride showed a lower percentage retention of calcium than did their pair mates receiving no fluorine. The average difference is +7.0 percent in favor of the control rat. The absolute amount of calcium retained daily is less for the fluorine-fed rat in all but two of the balance periods.

The percentage balance data for the rats receiving 0.0623 percent fluorine as sodium fluoride or calcium fluoride ap-

TABLE IV.—*Effect of fluorine as sodium fluoride and calcium fluoride on the percentage retention of calcium*

Period No.	Rat Nos.	Percent Ca retained		Difference
		Control rats	Fluorine-fed rats	
0.0106 percent fluorine as sodium fluoride				
1	1, 2	40.5	65.8	−25.3
2		79.4	75.9	+3.5
1	3, 4	43.5	53.0	−9.5
2		76.0	81.8	−5.8
1	5, 6	38.7	18.8	+18.9
2		61.2	55.5	+5.7
Average		56.5	58.4	−2.1
0.0313 percent fluorine as sodium fluoride				
1	7, 8	61.1	65.3	−4.1
2		45.8	44.7	+1.1
1	9, 10	62.9	58.3	+4.6
2		46.9	41.3	+4.6
1	11, 12	69.7	54.1	+15.6
2		42.6	54.8	−12.2
Average		54.8	53.1	+1.6
0.0313 percent fluorine as calcium fluoride				
1	19, 20	62.9	52.2	+10.7
2		38.6	48.0	−9.4
1	21, 22	66.1	68.9	−2.8
2		50.1	50.4	−0.3
1	23, 24	64.5	67.0	−2.5
2		49.5	52.1	−2.6
1	25, 26	60.9	65.4	−4-5
2		36.2	33.0	+3.2
Average		53.6	54.6	−1.0
Average for 0.0313 percent F		54.1	54.0	+0.1
0.0623 percent fluorine as sodium fluoride				
1	13, 14	58.3	44.3	+14.0
2		35.9	25.9	+10.0
1	15, 16	56.1	46.6	+9.5
2		57.0	22.1	+34.9
1	17, 18	39.5	33.5	+6.0
2		24.4	40.0	−15.6
Average		45.2	35.4	+9.8
0.0623 percent fluorine as calcium fluoride				
1	27, 28	26.5	38.4	−11.9
2		26.7	20.6	+6.1
1	29, 30	34.3	24.2	+10.1
2		28.1	18.6	+9.5
1	31, 32	32.3	29.5	+2.8
2		39.2	19.5	+19.7
1	33, 34	39.8	22.9	+16.9
2		24.5	21.7	+2.8
Average		31.4	24.4	+7.0
Average for 0.0623 percent F		37.3	29.0	+8.2

pear worthy of a statistical analysis. However, the data for the rats receiving lower levels of fluorine either as sodium fluoride or calcium fluoride do not indicate differences of any significance. Considering the case of the rats fed 0.0623 percent fluorine, the average difference in percentage retention of calcium for the same series of comparisons is +8.20 (favoring the control rats), with a probable error of 1.66 and a standard deviation of 2.47. The average difference is 3.32 times its standard deviation, and would occur, by chance only, once in 1,111 trials. It can be said with practical certainty that the level of 0.0623 percent fluorine has lowered the percentage retention of calcium of the fluorine-fed rats.

From these analyses of the data it appears (*a*) that neither a level of 0.0106 percent fluorine in the ration as sodium fluoride, nor a level of 0.0313 percent fluorine as either sodium or calcium fluoride has had an effect on the calcium balances of the rats; (*b*) that, with a probability equal to practical certainty, a level of 0.0623 percent of fluorine, either in the form of sodium fluoride or calcium fluoride, has lowered the percentage of the calcium intake that otherwise would have been retained by the experimental rats.

COMPOSITION OF THE HUMERUS AND FEMUR OF THE RAT AS AFFECTED BY INGESTION OF SODIUM FLUORIDE AND CALCIUM FLUORIDE

At the end of from 80 to 90 days, the rats were killed and the two humeri and the two femurs were dissected out. The flesh was removed from the bones as completely as possible, and the bones were dried for 2 days at 96°. The bones were extracted in a Soxhlet extraction tube with 95 percent alcohol for 18 hours and then with anhydrous ether containing 2.0 percent alcohol for another 18 hours. After the extraction, the bone residues were dried at 96° for 1 hour and weighed. They were ashed in silica crucibles in an electric muffle furnace for 3 hours and the percentage of ash calculated on the dry alcohol-ether extracted basis. Each sample of bone ash was dissolved *in toto* in 1:1 HCl, and transferred to a 250 cc. flask. The solution was made up to volume with distilled water, and aliquots of this solution were used for the determination of calcium and phosphorus. Calcium was determined by McCrudden's procedure for calcium in feces. Phosphorus was determined by the volumetric method (*15*), standardized sodium hydroxide being used to titrate the suspension of ammonium-phosphomolybdate precipitate.

From a study of table V in which the results secured with sodium fluoride appear, there seem to be no differences in the composition of the bones of fluorine-fed and control rats at the lowest level of fluorine intake. However, in all the pairs of rats used to test rations containing 0.0313 percent and 0.0623 percent of fluorine as sodium fluoride there is a slightly greater percentage of ash present, in both humerus and femur, for the fluorine-fed rat. The average difference for the femurs is +1.46 percent in favor of the fluorine-fed rat, and for the humeri +1.14 percent.

On considering the effect of calcium fluoride, as shown by the data of table VI, here again a slightly greater percentage of ash is present in the bones of rats fed fluoride than in those of their controls, in 11 of the 16 possible comparisons.

Although the results show a consistent effect of sodium fluoride at levels of 0.0313 percent and 0.0623 percent fluorine, a more definite significance may be attached to these results, if the data be interpreted statistically, since the number of trials is not large. The average difference between the ash contents of the humeri and femurs of the rats fed 0.0313 percent and 0.0623 percent fluorine as sodium fluoride, and those of their controls, is +1.305 percent, favoring the fluorine-fed rat. The standard deviation of the differences is 0.991 percent, and that of the average of the 12 differences (equal to $0.991 \div \sqrt{12}$), equals 0.285. The average difference is therefore 4.57 times its standard deviation, and hence with a degree of probability amounting to certainty it was the result of the directly imposed differential factor, namely, fluorine.

The feeding of calcium fluoride did not give as consistent results on the percentage of bone ash as did the feeding of sodium fluoride. It may be shown by statistical analysis that the idea on the ash contents of the bones cannot be interpreted positively as indicating an effect of calcium fluoride. The average difference in the percentage of ash in the humerus and femur of the rats receiving calcium fluoride as compared with their controls is only +0.265 percent favoring the fluorine-fed rat. The standard deviation of this average is 0.150. The average difference is therefore only 1.77 times its standard deviation. The probability of such a difference (or a larger one) occurring by chance alone is one in thirteen trials. In the case of the femurs alone, the comparison of test and control rats can scarcely be regarded as significant, although out of the eight comparisons six show a greater percentage of ash in the case of the fluorine-fed rat. The average difference here is +0.331 percent. With such a small number of comparisons the use of "Student's" method (*30*), devised for the analysis of small groups of paired data, is a fairer basis of interpretation than is the ordinary probability method, based upon the normal frequency curve. The probability as determined by "Student's" method (with $s = 0.543$, $z = 0.61$, and $n = 8$) is equivalent to odds of only 13 to 1 that the difference is a fluorine effect. It cannot be said, therefore, that calcium fluoride has affected the percentage of ash in the bones of the experimental rats. If such an effect is associated with the feeding of CaF_2 at the levels used in these experiments, it is of such a magnitude that its demonstration will require a considerably larger number of animals than were here used.

A study of table V shows that there is no consistent difference in the calcium content of the bone ash of the paired rats at the lowest levels of fluorine (NaF) intake. However, at the two higher levels there was a slightly smaller amount of calcium in the ash of the

TABLE V.—*Effect of fluorine as sodium fluoride on the composition of the bones of white rats* [1]

Rat No.	Femur				Humerus			
	Ash	Ca in ash	P in ash	Ca:P	Ash	Ca in ash	P in ash	Ca:P
0.0106 percent fluorine								
1	61.81	35.98			59.44	34.19		
2	60.17	34.14	17.92	1.90	63.69	34.27	17.96	1.91
3	57.11	35.53	18.67	1.90	56.51	30.01	17.77	1.69
4	60.21	35.40	17.99	1.97	60.11	31.46		
5	63.17	34.05	17.88	1.90	63.27	31.46	18.36	1.71
6	63.27	33.12	18.21	1.82	60.24	30.93	18.66	1.66
0.0313 percent fluorine								
7	65.29	33.72	18.81	1.79	65.07	31.92	18.31	1.74
8	63.01	35.15	18.22	1.93	63.49	32.14	17.91	1.79
9	65.12	33.43	18.35	1.82	64.25	30.49	18.91	1.61
10	62.07	34.11	18.37	1.86	61.71	33.45	17.99	1.86
11	63.69	33.66	18.53	1.82	63.50	33.03		
12	63.41	34.66	18.54	1.87	63.11	32.30	17.61	1.83
0.0623 percent fluorine								
13	63.40	34.85	18.62	1.87	65.37	31.56		
14	62.83	34.63	18.63	1.86	63.48	32.90		
15	62.50	33.35	18.71	1.78	62.79	30.72	18.11	1.70
16	61.91	34.39	17.87	1.92	62.34	33.16	18.90	1.75
17	63.95	33.75			63.83	30.94		
18	61.94	34.69	18.07	1.92	63.80	32.48	18.34	1.77

[1] The results are expressed in percent.

TABLE VI.—*Effect of fluorine as calcium fluoride on the composition of the bones of white rats* [1]

Rat No.	Femur				Humerus			
	Ash	Ca in ash	P in ash	Ca:P	Ash	Ca in ash	P in ash	Ca:P
0.0313 percent fluorine								
19	63.49				64.03	32.50	17.44	1.86
20	63.34	34.54	18.15	1.90	63.02	32.93	17.84	1.84
21	62.26	34.28	18.49	1.85	62.80	31.77	18.17	1.75
22	62.96	36.49	18.29	1.99	62.99	32.39	18.32	1.77
23	63.26	34.67	18.43	1.88	64.11	33.02	18.35	1.80
24	62.42	34.94	18.63	1.87	63.33	34.10	18.33	1.86
25	62.94	33.08	18.66	1.77	62.94	34.29	18.58	1.84
26	62.73	32.90	18.80	1.75	63.01	32.20	18.27	1.76
0.0623 percent fluorine								
27	63.86	34.43	18.11	1.90	62.56	34.07	18.02	1.89
28	64.10	36.26	18.59	1.95	63.52	33.84	17.92	1.89
29	64.27	35.16	18.35	1.92	62.71	33.78	18.75	1.80
30	63.47	35.56	18.57	1.91	62.54	33.15	18.56	1.79
31	63.88	34.69	18.28	1.90	64.24	32.54	18.25	1.78
32	63.00	35.30	18.49	1.91	63.40	33.36	18.78	1.78
33	62.92	34.65	18.38	1.88	63.07	34.37	18.71	1.84
34	62.21	36.72	18.46	1.99	63.06	33.22	18.72	1.77

[1] The results are expressed in percent.

sodium fluoride-fed rats, in 10 of the 12 comparisons of the humerus and femur.

The average difference for the femurs is 0.81 percent in favor of the control rat; for the humeri of the same rats the average difference, possessing the same significance, amounts to 1.29 percent.

Of the twelve comparisons possible, both femurs and humeri being included, the average difference between pair mates in the percentage of calcium in the ash is −1.05, the negative sign indicating a lower calcium content in the case of the fluorine-fed rat. The standard deviation of this average difference is 0.298. The average difference is 3.53 times its standard deviation. An average difference of this size or greater would occur as a result of chance only, once in 2381 trials. Its significance as a fluorine effect can hardly be doubted.

The effect of calcium fluoride is shown by the data in table VI; there is a smaller percentage of calcium present in the ash of the femurs of the fluorine-fed rat in all but one of the seven possible comparisons between pair mates. The humeri of the same rats, however, show less calcium in the ash for the fluorine-fed rats in four pairs and more calcium in the remaining four pairs. Thus, with reference to the percentage of calcium in the ash of the bones, as well as to the percentage of ash in the bones, it is evident that the feeding of calcium fluoride has given less consistent results than the feeding of sodium fluoride indicating a less pronounced effect upon bone composition. The data concerning the percentage of calcium in the ash of the femurs may warrant an analysis by statistical methods.

The significance of the differences obtained may be assessed with a greater degree of certainty by "Student's" method, than is possible by inspection only. The average difference between pair mates in calcium content for the seven possible comparisons is −1.030 percent, the negative sign indicating a smaller average concentration of calcium in the bone ash of the fluorine-fed rats as compared with their pair mates. The standard deviation of the seven differences is 0.905. The average difference divided by the standard deviation equals 1.14 ("Student's" z), a value of z which for $n = 7$ indicates that an average difference of 1.03 percent or larger would occur but once in 63 trials, if uncontrolled factors alone determined the results of this experiment. This statistical result represents a fairly high degree of certainty that other than chance factors were operating. Odds of 1 in 30 or 1 in 50 are generally regarded as practically conclusive in biological experiments. It may be concluded, therefore, that the feeding of calcium fluoride at the levels used in these experiments (equivalent to 0.0313 and 0.0623 percent of fluorine) has lowered the normal content of calcium in the femurs of the experimental rats, although no such effect is evident on the composition of the humeri. Apparently the humerus is less susceptible to dietary fluorine than the femur.

There is no indication that fluorine, either as sodium fluoride or calcium fluoride, had any effect on the phosphorus content of the bone ash. Of eight possible comparisons between pair mates of the results of feeding the two higher levels of sodium fluoride (see table V), four show a greater amount of phosphorus in the ash of the fluorine-fed rat and four a lesser amount.

The same even distribution of differences in the phosphorus content of the ash occurs for the rats fed calcium fluoride (see table VI), the humeri only being considered. In the case of the femurs, however, in six of the seven possible comparisons between pair mates, the fluorine-fed rat shows the smaller percentage of phosphorus. The average difference for these seven comparisons is −0.161 percent and their standard deviation is 0.188 percent. The average difference is only 0.86 times the standard deviation, which, by "Student's" method of interpretation, indicates a chance occurrence of once in 25 trials. It cannot be said with certainty that the amount of phosphorus in the bone ash has been modified by the presence of either calcium fluoride or sodium fluoride in the ration.

Differences in the calcium to phosphorus ratios in the bone ash for the rats fed sodium fluoride as compared with the same ratios for their pair mates show, in all but one of the eight possible comparisons, a lower value for the fluorine-fed rat, indicating a smaller amount of calcium in proportion to phosphorus in the bone. The average difference for the eight comparisons is 0.0887 with a standard deviation of 0.0774. By "Student's" method of analysis this average difference would be expected to occur by chance but once in 102 trials. The results may be regarded as indicating, therefore, a definitely smaller concentration of calcium as compared with phosphorus in the bones of the fluorine-fed rats.

It cannot be said that calcium fluoride has had any influence on the calcium to phosphorus ratio. Of the seven comparisons of the femurs there are three positive differences and four negative differences. Of eight such comparisons based on the humeri, in two cases no difference exists, in four cases the difference is positive, in two negative.

Effect of Sodium Fluoride and Calcium Fluoride on Development of the Teeth of Rats

McCollum, Simmonds, Becker, and Bunting (*17*) produced marked changes in the development of the teeth of rats by feeding a ration containing 0.0226 percent fluorine as sodium fluoride. In two rats the effect was apparent within 77 and 97 days respectively. The other rats were on experiment from 201 to 338 days. Second generation rats were fed from 161 to 179 days and showed overgrown incisors. Schulz and Lamb (*25*) fed the same level of sodium fluoride (0.0226 percent fluorine) without effect (for how long is not stated). At a level of 0.0452 percent fluorine as sodium fluoride the teeth of the rats fed by Schulz and Lamb became abnormal.

The rats in the present experiment were fed from 80 to 90 days. No visible effects were noted in the case of those rats receiving 0.0106 percent fluorine as sodium fluoride. Two rats out of three fed 0.0313 percent fluorine as sodium fluoride showed the usual abnormal overgrowth of the upper incisors, while the lower incisors are worn down excessively. The teeth of all the rats receiving 0.0313 and 0.0623 percent of fluorine as calcium fluoride showed the characteristic abnormal development.

It was noted that the teeth of the fluorine-fed rats, besides showing abnormal growth, did not have the peculiar orange tint on the anterior surface characteristic of normal teeth, but were generally a dull white in color.

SUMMARY

The deleterious effects noted in the literature as a result of the use of raw rock phosphate as a mineral supplement have been attributed by investigators generally to its fluorine content. An attempt has been made, therefore, to study the effect of fluorine on the rate of gain, the calcium balance, and the bone and tooth development of the growing rat.

The results of feeding fluorine to rats indicate that either calcium fluoride or sodium fluoride in the ration at levels of 0.0313 percent and 0.0623 percent fluorine inhibit growth, entirely aside from any effect on food consumption. The higher level of sodium fluoride appears to have lowered the food consumption of the rats also, but the corresponding level of calcium fluoride apparently had no such influence.

The calcium balance of the rats was not affected by either sodium fluoride or calcium fluoride at levels of 0.0106 percent and 0.0313 percent fluorine in the ration. The results indicate with a probability equal to practical certainty, that a level of 0.0623 percent of fluorine in the form of either calcium fluoride or sodium fluoride, lowered the percentage of calcium that otherwise would have been retained by the experimental rats.

The composition of the bones of growing rats was distinctly affected by sodium fluoride at levels of approximately 0.03 per cent and 0.06 per cent fluorine. A consistent increase, averaging 1.30 percent, was evident in the ash content of the bones, and a statistically significant, though not as consistent, a decrease in the calcium content of the ash, averaging 1.05 percent. The phosphorus content of the ash was not significantly affected, but the ratio of calcium to phosphorus in the ash was depressed.

The results indicate at least a tendency for the same levels of fluorine as calcium fluoride to increase the precentage of ash in the bones. A significant depression of calcium in the ash of the femur, but not of the humerus resulted from calcium fluoride feeding. No definite decrease in the percentage of phosphorus in the ash of the bones accompanied the decrease in calcium occasioned by the calcium fluoride feeding. The Ca:P ratio was not affected.

It may be concluded, therefore, that fluorine, especially when consumed in the more soluble form of the sodium salt, may cause the deposition of an apparently abnormal constituent in the bones, or an abnormal deposition of a noncalcium constituent, possibly a fluoride of a mineral other than calcium, as evidenced by an increase in the percentage of ash in the bones above the normal. There is also an interference with the deposition of calcium in the bones, brought about by feeding fluorine, a result in agreement with the calcium balance studies, previously described.

The peculiar effect of fluorine on the development of the teeth of rats was again demonstrated in this experiment. A level of 0.03 percent and 0.06 percent fluorine in the form of sodium fluoride or calcium fluoride brought about the characteristic abnormalities. Contrary to the results of bone analysis, the insoluble calcium salt was as effective as the soluble sodium salt, in bringing about a change in tooth structure.

Note—After page proofs of this article had been received it was discovered that a definite statement of the calcium supplements added to the basal ration in this experiment had been omitted through oversight. Synthetic mixtures of tricalcium phosphate and sodium fluoride and tricalcium phosphate and calcium fluoride were prepared to contain approximately 4.0 percent fluorine, the approximate amount of fluorine which is present in commercial grades of rock phosphate. The synthetic mixtures were added to the basal ration to supply the desired percentages of fluorine. Control rations were prepared by adding tricalcium phosphate to the basal ration in such amounts that the calcium contents of control and test rations were approximately the same. The minerals added to the ration replaced an equal amount of starch. All rations were analyzed for calcium.

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EFFECT OF FLUORIDES ON SALIVARY AMYLASE

By F. J. McClure

Amylolytic enzymes have been reported to be more active in the presence of fluorides (*1, 2, 3*), to remain unaffected by fluorides (*4, 5, 6, 7*), or to be inhibited in their reactions (*5, 6, 8, 9, 10*). According to Clifford (*5, 6*), the fluorides, KF and NH_4F, were found to cause a marked inhibition of pancreatic and salivary amylase, whereas NaF was inert, up to a concentration of 0.5 M. The contradiction in the results cited above appears to be due to a failure to maintain certain optimum conditions as regards pH and activating electrolyte, known to be required for normal amylolytic enzyme action. The work of Sörensen (*11*), Myrbäck (*12*), and Sherman, Thomas and Baldwin (*13*) demonstrated the extreme sensitivity of amylolytic reactions to slight variations in pH. The optimum pH for salivary amylase in the presence of chloride activation is about pH 6.7. A change to pH 6.0 or 7.5, for example, may inhibit the activity of amylase as much as 25 percent (*12*).

Sherman, Caldwell, and Adams (*14, 15*), studied the optimum pH at which various electrolytes activate salt-free pancreatic amylase. NaF activates salt-free amylase up to 24 percent of the total activation produced by sodium chloride, the optimum pH for 0.10 M, 0.20 M, and 0.30 M NaF activation being 6.3–6.7, 6.6–6.8, and 6.6–6.8, respectively. Myrbäck (*12*) worked with purified amylase also. In the presence of 0.0015 N NaCl he found that 0.03 N KF was without effect on amylolytic action.

The results presented in this paper give no indication of an effect on salivary amylase of quantities of NaF, KF, NH_4F and Na_2SiF_6 which are undoubtedly physiologically excessive and which are greater than any quantities of fluoride actually encountered under conditions of chronic endemic fluorosis (*16*). A reaction pH of approximately 6.6 was maintained by means of a phosphate buffer, and activation of the enzyme was assured by adding sodium chloride to the substrate prior to testing the effect of fluoride.

The procedure for determining amylolytic activity was as follows: A soluble starch substrate was prepared consisting of 25 cc. of a 1 percent solution of soluble starch (Mallinckrodt's soluble starch was used, the solution being boiled for 3 minutes), 1 cc. of a KH_2PO_4–Na_2HPO_4 buffer (pH 6.6), and 1 cc. of a 1 percent solution of sodium chloride. The volume was made up to 50 cc. in a 50-cc. glass stoppered digestion cylinder and brought to a temperature of 37.5° C. in a constant temperature oven. One cubic centimeter of a 1 to 10 dilution of stimulated saliva was added and the reaction allowed to proceed for exactly one-half hour at 37.5° C. The reaction was stopped by adding 2 cc. of normal HCl, and the contents of the digestion cylinder transferred to an Erlenmeyer flask. The acid was just neutralized with dilute alkali. Total reducing sugars were then determined by titration with standard iodine and thiosulfate, following the procedure recommended by Kline and Acree (*17*). The results are recorded as milligrams of maltose produced.

Two methods for testing the possible effects of fluoride were followed. In one (table 1) the saliva was diluted 1 to 10 with the various fluoride solutions and allowed to stand 1 hour in the cold before measuring enzyme activity, and in the other (table 2), the fluoride solutions were added to the substrate directly, before adding 1.0 cc. of a 1 to 10 water-dilution of saliva. The data presented in table 1 show that concentrations of fluorides varying from 1.7 to 8,550.0 p. p. m. of fluorine in the diluted salivas were without effect on the subsequent activity of the enzyme. Where NH_4F and Na_2SiF_6 were present in high concentrations (table 1) the reactions were not properly buffered by 1 cc. of the usual phosphate buffer (pH 6.6). Inhibition of enzyme activity in these cases is due to a modified pH, but by proper buffering these fluoride salts also may be shown to be innocuous at these levels (table 1).

The results presented in table 2 show no effect of NaF, KF, NH_4F, and Na_2SiF_6 in concentrations equaling 0.76, 7.6, 76.0, and 760.0 parts per million of fluorine. Sodium fluoride and KF were inert up to concentrations equal to 3,800 parts per million of fluorine. The inhibition caused by NH_4F and Na_2SiF_6 at fluorine concentrations equaling 3,800 parts per million of fluorine was not investigated further, since such quantities are in great

Table 1.—*Effect of fluorides on salivary amylase. Saliva diluted 1 to 10 with fluoride solutions and allowed to stand 1 hour in cold before testing enzyme activity*

Fluoride	F in diluted saliva (p. p. m.)	F present during reaction (p. p. m.)	pH of reaction		Reducing sugars calculated as maltose (mg.)		Enzyme activity compared as percent of control	
Saliva sample			F. J. M.	W. S. M.	F. J. M.	W. S. M.	F. J. M.	W. S. M.
Control	0.0	0.0	6.5	6.6	107.8	99.6	100.0	100.0
NaF	8550.0	171.0	6.6		109.1	99.2	101.2	100.4
Do	1710.0	34.2			108.3	101.3	100.5	101.7
Do	171.0	3.4			105.8	102.9	98.1	103.3
Do	17.1	.3			102.9	99.4	95.5	99.8
Do	1.7	.03			105.5	100.4	97.9	100.8
Control	0.0	0.0	6.6	6.6	114.4	94.9	100.0	100.0
KF	8550.0	171.0	6.6		115.6	94.8	101.0	99.9
Do	1710.0	34.2			110.1	96.9	96.2	102.1
Do	171.0	3.4			113.3	97.4	99.0	102.6
Do	17.1	.3			110.5	96.8	96.6	102.0
Do	1.7	.03			112.0	96.7	97.9	101.8
Control	0.0	0.0	6.6	6.6	106.8	93.5	100.0	100.0
NH_4F	8550.0	171.0	5.8	5.6	94.5	53.8	88.5	57.5
Do	1710.0	34.2		6.4	108.3	93.9	101.4	100.4
Do	171.0	3.4			104.5	97.7	97.8	104.4
Do	17.1	.3			104.6	96.8	97.9	103.5
Do	1.7	.03			101.8	95.2	95.3	101.8
Control	0.0	0.0	6.6	6.6	105.5	92.9	100.0	100.0
Na_2SiF_6	8550.0	171.0	3.7	3.6	12.2	12.9	11.6	13.9
Do	1710.0	34.2	3.8	3.8	16.7	17.0	15.8	18.3
Do	171.0	3.4		6.3	103.7	91.9	98.3	98.9
Do	17.1	.3			104.8	92.5	99.3	99.5
Do	1.7	.03			108.2	92.2	102.5	99.2
Control	0.0	0.0	6.6	6.8	97.0	96.6	100.0	100.0
Na_2SiF_6	8550.0	171.0	6.5	6.9	92.6	95.7	95.4	95.9
Do	1710.0	34.2	6.6	7.0	98.2	94.1	101.2	94.1
NH_4F	8550.0	171.0	6.5		104.1	99.0	107.3	102.4

TABLE 2.—*Effect of fluorides on salivary amylase. Fluorides present during enzyme-substrate reaction*

Fluoride	F present during reaction (p. p. m.)	pH of reaction		Reducing sugars calculated as maltose (mg.)		Enzyme activity compared as per cent of control	
Saliva sample	....	F. J. M.	W. S. M.	F. J. M.	W. S. M.	F. J. M.	W. S. M.
Control	0.0	6.6	6.6	101.7	101.3	100.0	100.0
NaF	3,800.0	6.5	....	94.7	87.6	90.4	86.5
Do	760.0	6.5	6.4	103.5	95.6	98.9	94.4
Do	76.0	6.6	6.6	97.9	99.4	90.9	98.1
Do	7.6	6.6	6.6	107.8	100.8	100.1	99.5
Do	.76	6.6	6.6	103.7	97.4	98.3	96.2
Control	0.0	6.6	6.6	112.4	99.2	100.0	100.0
KF	3,800.0	....	6.9	96.8	100.5	86.1	101.3
Do	760.0	....	6.4	104.2	94.9	92.7	95.6
Do	76.0	....	6.5	109.9	97.6	97.8	98.3
Do	7.6	....	....	110.8	98.7	98.6	99.4
Do	.76	....	....	111.2	95.5	98.9	96.2
Control	0.0	6.6	6.6	110.3	99.9	100.0	100.0
NH_4F	3,800.0	6.1	....	24.6	29.2	22.3	29.2
Do	760.0	5.4	5.5	41.3	35.9	42.0	35.9
Do	76.0	6.3	....	110.6	86.6	100.2	86.7
Do	7.6	6.6	....	111.3	98.1	100.9	98.2
Do	.76	....	....	112.9	100.3	102.3	100.4
Control	0.0	6.6	6.0	108.3	98.9	100.0	100.0
Na_2SiF_6	3,800.0	3.7	....	0.0	0.0	0.0	0.0
Do	760.0	3.6	3.6	0.0	0.0	0.0	0.0
Do	76.0	3.6	3.6	18.3	15.3	16.9	15.8
Do	7.6	5.5	....	84.0	74.9	77.6	77.3
Do	.76	6.4	....	108.5	97.2	100.1	100.3
Control	0.0	6.6	....	110.1	96.8	100.0	100.0
Na_2SiF_6	760.0	6.5	6.2	102.8	84.4	93.4	87.2
Do	76.0	6.5	....	95.3	99.0	86.6	102.3
Do	7.6	....	6.8	102.8	96.8	93.4	100.0
Control	0.0	6.6	....	116.2	93.8	100.0	100.0
NH_4F	760.0	6.8	....	108.4	87.2	93.3	93.0

excess physiologically. Substrates containing Na_2SiF_6 in concentrations, equaling 7.6, 76.0, and 760.0 parts per million of fluorine as well as substrates containing NH_4F in a concentration equal to 760 parts per million of fluorine required special buffering before normal amylolytic action was obtained.

EFFECTS OF FLUORIDES IN DRINKING WATER

The presence of fluorides in drinking water is the cause of endemic mottled enamel (*16*), and there may be other toxic effects resulting from the ingestion of fluorides. According to the enzyme studies presented above, unabsorbed fluorides in the drinking water will not affect the reaction of salivary amylase in the human system. However, there remained the possibility of a physiological effect of fluorides absorbed from drinking water and food on the salivary amylase as secreted. The following data throw light on this latter question.

In connection with a recent dental survey conducted by Dean et al. (*18*), saliva specimens from a group of school children whose drinking water contained on the average 1.8 parts per million of fluorine (Galesburg, Ill.) were available for determination of amylolytic activity. These specimens were compared with a number of other specimens collected under similar conditions from children whose drinking water contained no fluorine (Quincy, Ill.). The two groups of salivas were packed in ice and were received at the laboratory in Washington, D.C., at temperatures of 7° C. and 10° C., respectively. Amylase was determined according to the method outlined above, except that Merck's soluble starch, according to Lintner, was used instead of the Mallinckrodt product. Slightly more maltose resulted from the use of the Merck starch. A total of 63 specimens of saliva from children living in Quincy, Ill., averaged 105.9 ± 5.2 mg. of maltose, as compared with an average of 108.7 ± 3.1 mg. of maltose for 82 specimens from children living in Galesburg, Ill. These results include all salivas from each group, although the individual data indicate that a number of salivas from each group may have deteriorated after the time of collection in spite of the low temperature maintained. Figures for total maltose, which were somewhat less than 90 mg., were thought to be evidence of a loss of amylolytic activity following collection of the saliva. This may or may not be the case. Only a limited number of data are available from which to determine what variations may occur normally among a group of individual salivas. Among the above data, 17.4 percent of the results obtained on the samples of saliva from Quincy showed less than 90 mg. of maltose, and 14.6 percent of the samples from Galesburg gave less than 90 mg. of maltose (table 3). An upper limit of 137 mg. of maltose was obtained in one saliva sample. The following table gives information regarding the general consistency of the data.

The means of these distributions, i.e., 105.9 ± 5.2 mg. for Quincy and 108.7 ± 3.1 mg. for Galesburg, show no statistically significant differences. It may be said with reasonable assurance that fluoride ingestion, brought about by the use of a domestic water supply containing approximately 1.8 parts per million of fluorine, does not change the final amylolytic activity of the saliva secreted under these conditions

CONCLUSIONS

The fluorides, NaF, KF, NH_4F, and Na_2SiF_6, were found to have no effect on the activity of salivary amylase in concentrations varying from 1.7 to 8,550 parts per million of fluorine present in 1 to 10 dilutions of salivas which stood for 1 hour in the cold prior to testing amylolytic property. The same fluorides, when present in the enzyme-substrate mixture during the digestion period, in concentrations varying from 0.76 to 760 parts per million of fluorine in the substrate, had no final effect on enzyme activity. The salivas of school children whose drinking water contained an average of 1.8 parts per million of fluorine showed no differences in amylolytic action from a similar group of salivas obtained under similar conditions from school children whose drinking water was free from fluoride.

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TABLE 3.—*Distribution of the saliva specimens by cities according to maltose producing activity expressed in milligrams*

Maltose (mg.)	Galesburg		Quincy	
	Number	Percent of total	Number	Percent of total
(45)-50	0	0.0	4	6.3
50-90	12	14.6	7	11.1
90-100	5	6.1	6	9.5
100-110	16	19.5	11	17.5
110-120	27	32.9	22	34.9
120-130	16	19.5	7	11.1
130-(137)	6	7.3	6	9.5
	82	----------	63	----------

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FLUORIDES IN FOOD AND DRINKING WATER (A Comparison of Effects of Water-Ingested Versus Food-Ingested Sodium Fluoride)

By F. J. McCLURE

INTRODUCTION

Observations on human fluorosis in most instances limit its manifestation to an endemic hypoplasia generally of the permanent teeth known as mottled enamel. In more severely affected teeth a characteristic brown stain is not infrequently a secondary complication. Epidemiological studies show that the development of endemic mottled enamel is limited to areas where a domestic water containing toxic quantities of fluorides is used.

The results of analysis of a number of common foods indicate the occurrence of fluorine in some foods as a natural constituent. The addition of inorganic fluorine to certain foods as a result of spraying with fluorine-bearing substances for insect control also represents a possible source of fluorine which may be ingested in the food. In the production of human fluorosis, the part played by any naturally occurring fluorine compounds contained in foods and by any fluorine which spray residues may contribute to certain foods remains largely unknown.

The production, due to fluorosis, of mottled enamel of the permanent teeth, may occur only if certain minimum quantities of fluorine are ingested regularly during the period of development of the crowns of the teeth. This period is from birth to 8 years of age (excepting the third molars). In general, those foods which appear to contain toxic quantities of fluorine naturally or which are treated with fluorine-bearing sprays during production do not ordinarily constitute an appreciable portion of the diet of infants and children. Even though a particular food may compare with toxic drinking water in fluorine content, total quantities of food and drinking water actually consumed regularly and daily will determine the ultimate effects.

In addition to the question of total quantities of fluorine provided by foods as compared with drinking water, it may be surmised that the naturally occurring fluorine-bearing substances found in foods may not be as toxic as known pure inorganic fluorine compounds or inorganic fluorine contained naturally in certain drinking waters. Finally, there exists the possibility that the mode of ingestion might differentiate the effects of fluorine compounds contained in drinking waters from effects of similar compounds contained in foods. In the case of fluorine contained in water used for drinking and cooking purposes, most of the fluorine ingested is in the water drunk; an additional quantity is added to the food during cooking. In the case of fluorine contained in foods, all of the fluorine taken into the body is by way of the food eaten.

This experiment furnishes information as to whether a fluorine-bearing compound as represented by sodium fluoride, when consumed in the drinking water will show the same degree of toxicity as when ingested in the food.

One group of workers has observed that a given concentration of sodium fluoride in the drinking water produces a more toxic reaction than the same concentration of sodium fluoride in the diet (*1, 2*). These tests compared the rate of growth of groups of rats receiving 500 p.p.m. of sodium fluoride in the diet with the growth of similar groups receiving 500 p.p.m. of sodium fluoride in the drinking water. Among a group of four rats studied (*2*) two rats receiving sodium fluoride in the drinking water died by the end of the 10th day; the other two showed reduced rate of growth. All four rats in a group on 500 p.p.m. of sodium fluoride in the diet grew at a practically normal rate. Changes in the teeth of this latter group were not as severe as those noted in the group receiving the 500 p.p.m. of sodium fluoride in the drinking water. In the earlier experiment (*1*) a group of six rats was given 500 p.p.m. of sodium fluoride in the drinking water and "the animals on this water gained weight for the first 3 days and then lost weight during the next week, followed by a rapid and continuous gain in weight, with the exception of one rat which died in 11 days from the beginning of the experiment." An attempt was made to repeat this experiment (i.e., using 500 p.p.m. of sodium fluoride in water) with a total of 20 rats. Sixteen rats died within 11 days. The four that survived the acute toxic effects of the fluoride grew well but showed the same chalky white brittle teeth that were seen in the first group. Ad libitum feeding was allowed throughout these experiments.

It was concluded that "500 p.p.m. of sodium fluoride in the drinking water produced a relatively greater toxicity than 500 p.p.m. of sodium fluoride in the diet, under the conditions of this experiment." It was suggested by the authors that this result was probably owing "to differences in the total quantity of sodium fluoride ingested, the more complete absorption, to a more rapid rate of absorption, or to other factors not definitely determined."

In a series of experiments covering a 6-weeks' growth period in young rats, Smith and Leverton (*3*) tested several fluorine compounds dissolved where possible in distilled water, which was used as drinking water, or they dissolved or mixed the fluorine compounds in distilled water and introduced the solutions directly into the throats of the rats with a medicine dropper. In other experiments the same compounds were fed separately and in other series definite weighed quantities were mixed in solid form with a small quantity of the basal diet. Whether or not the method of administering the fluorine substances caused any difference in effects noted is not disclosed from the data as reported, nor from the authors' discussion. Apparently no pronounced variations attributable to food versus water ingestion were observed. At least, as the authors state, "no great difference in toxicity of fluorine in different combinations," was observed "when the amount required to produce the first discernible effects upon the teeth is the basis for comparison."

Some effects of food on alcohol absorption may be cited in this connection, having in mind the fact that the quantities tested produced what may be regarded as a condition of acute toxicity. In experiments with dogs, the rate of absorption, as determined by the alcoholic content of the blood at regular intervals, was definitely reduced by a meal of bread and milk taken before the alcohol (*4*). The most effective food taken was milk and the action in this case seemed to be dependent to some extent on its fat content. An interesting observation of this same experiment is the stimulating effect of water on the absorption of alcohol. A drink of water some hours before the alcohol caused a more rapid absorption of the alcohol, just as the fluid of a first drink of alcohol can act as a stimulus to the absorption of the alcohol of a second drink. Since alcohol requires no digestion for absorption, the effect of water in this case would appear to be unrelated to the observed stimulating effect of water on the secretion of gastric juice (*5, 6*). In this case, therefore, as suggested by Mellanby (*4*), water may have a specific stimulating effect on the cells of the intestinal mucous membrane involved in absorption. It is impossible to apply without certain reservations, however, these effects produced on alcohol absorption, to fluorides and other mineral or organic food nutrients. Alcohol is rapidly absorbed from the stomach, for example, whereas under ordinary conditions soluble inorganic salts (fluorides in low concentrations presumably may be included among such salts) are practically unabsorbed before reaching the small intestine. It should be emphasized that the effects observed with alcohol are referable almost entirely to variable rates of absorption.

The presence of food in the gastrointestinal tract shows no pronounced effect on the total absorption of lead, although the data in this regard are limited. Cats fed lead acetate in milk as compared with cats given lead acetate in water 12 or 20 hours after food showed but slight differences in total quantities of lead retained (*7*); if any difference is indicated in these data, it is in the direction of a slightly greater retention from water-ingested lead.

In the case of fluorine, as well as most other elements or individual food nutrients, few direct attempts have been made to determine the effect of food, per se, in the alimentary tract on the absorption of subtoxic quantities. In feeding experiments the substance studied is added to the diet and not to the drinking water. Dietary factors, so far as they are known, which are involved in the process of mineral absorption are, generally speaking, not subject to variations which might be attributed to food versus water ingestion of the mineral in question. However, it should be pointed out that, with the exception of calcium and phosphorus and perhaps iron, information regarding factors associated with the assimilation of mineral nutrient elements is largely unavailable, and, with respect to fluorine ingested in extremely small subtoxic quantities such as afford no symptoms of inanition or inhibited growth, the problem is perhaps largely one of total absorption and assimilation. As has become evident from this and previous experiments, the bones and the teeth become the main fluorine depositories.

PLAN OF EXPERIMENT

The experiment as planned studies fluorosis in the growing rat as produced by the ingestion of sodium fluoride. Three young growing rats weighing from 50 to 60 gm., of the same sex, and littermates, constituted a feeding group. Each rat of a group was fed separately the same quantity of food daily, the rat consuming the least quantity on a previous day determining the current day's allotment. One rat of each group served as a control and received distilled water and the basal diet. The second, or so-called water-fluoride rat, received drinking water containing a definite quantity of sodium fluoride and the basal diet. The third, or food-fluoride rat, received distilled water and the basal diet to which was added each day a quantity of sodium fluoride equal to the sodium fluoride consumed the day previous by the water-fluoride rat of the group. This allotment of sodium fluoride was made in cubic centimeters of a solution of sodium fluoride 10 times the concentration of the corresponding fluoride drinking water. The number of cubic centimeters of solution thus added to the ration of the food-fluoride rat was matched by an equal number of cubic centimeters of distilled water added to and similarly mixed with the day's ration of the other two rats of the group.

In one series of experiments, the food-fluoride rat consistently received a ration that contained 0.06 percent fluorine in the form of added sodium fluoride. The water-fluoride rats of this series received varying quantities of sodium fluoride in their drinking water, depending on their tolerance of fluoride dissolved in drinking water at these high levels.

This method of control calls for an accurate estimate of the quantity of water drunk. To accomplish this, a water fountain (see fig. A) was devised which corrected losses by spilling and evaporation, and provided for a satisfactory measure of the water consumed. The entire unit as shown in the figure was hung on the door of the cage over an opening cut in the cage to permit access to the fountain. The unit was weighed each day. The loss in weight equalled the water drunk by the rat plus evaporation from the exposed water surfaces. Every third cage was provided with a duplicate watering unit, which was not accessible to the rat. The loss in weight of this control unit provided a correction factor for evaporation losses. Any water spilled by the drinking rat fell into the cup and thus represented no loss in weight to the unit.

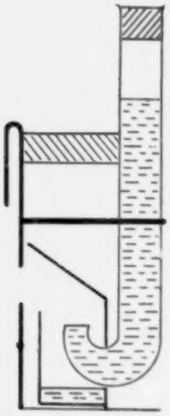


FIGURE A.—*Water fountain, which provides for an accurate measure of water intake.*

The evaporation losses of the control watering-unit and the units in use by the rat were subject to slight variations due to surrounding temperature and air current differences and to slight variations in dimensions of the fountains and cups. These differences were negligible and the device is regarded as affording a highly accurate measure of water consumption. All glass containers holding fluoride solutions received an inside coating of paraffin.

No attempt was made to purify the basal ration of fluorine. The ration contained ingredients as follows: casein, 20 percent; cottonseed oil 3 percent; cod liver oil, 2 percent; dried brewer's yeast, 5 percent; corn starch, 66 percent; Osborne-Mendell salt mixture, without the addition of sodium fluoride, 4 percent. Where sodium fluoride was added directly to the ration it replaced an equal quantity of starch. The ration analyzed 0.49 percent Ca, 0.53 percent P, and 3.2 percent N. It contained on the average approximately 4 p.p.m. of fluorine.

Four series of experiments were completed, involving 45 rats in all. The first series consisted of 5 groups of rats in which the food-fluoride rat of the group received 600 p.p.m. of fluorine in its ration regularly, while the water-fluoride rat was given sodium fluoride drinking water at a level in keeping with the rat's ability to survive. These quantities varied from 80.4 to 316.6 p.p.m. of fluorine. A second series of rats consisted of 4 groups. The water-fluoride rat received 90.4 p.p.m. of fluorine as sodium fluoride in its drinking water, the food-fluoride rat having corresponding intake-quantities added to its ration daily. The water-fluoride rats of the third series received 45.2 p.p.m. of fluorine in their drinking water; identical intake-quantities were added to the rations of the food-fluoride rats. In a fourth series, a level of 22.6 p.p.m. of fluorine in the drinking water was tested as above. Perhaps it should be mentioned that the experiment would have been more adequately controlled for the purpose of demonstrating an effect of food ingestion versus water ingestion, had the periods of eating and drinking been widely separated. Certainly a maximum effect would be demonstrated by withholding water for a considerable length of time after eating. However, conditions in practice were more nearly duplicated by allowing ad libitum eating and drinking so far as time was concerned.

In the first series the rats were fed for 10 weeks, the other groups for 7 weeks. The rats were weighed daily for the first and last 3 days and every other day during the remainder of the experiment. At the end the rats were killed by gas, the digestive tract emptied of food residues, and the empty weight determined. The heads were cut off and the lower incisor teeth photographed. The entire bodies were autoclaved at 15 pounds pressure for about three-quarters of an hour. The incisor teeth and humeri, tibiae, and fibulae were removed and all adhering tissue returned to the body sample. The body sample placed in a weighed porcelain dish was dried first in a current of warm air and dried finally in an oven at 90°C. The sample was ashed by first charring at a low temperature, allowing to cool, moistening with 5 percent magnesium acetate (*8*) and drying thoroughly. Ashing was then completed at 500° C. The weight of ash was determined, and after pulverizing in an agate mortar was placed in a sample bottle for fluorine analysis.

The bone samples and incisor teeth were broken into small pieces, and extracted with alcohol and ether, for periods of 12 hours for each solvent. The sample was placed in a weighed porcelain crucible, dried to constant weight at 90° C. and the weight determined. Ashing was completed in the muffle furnace at 500° C., and the weight of ash determined. After finely pulverizing in an agate mortar the samples were placed in bottles preparatory to fluorine analysis.

In order to obtain data necessary for the calculation of a fluorine balance, six stock rats weighing from 50 to 60 gm. were killed and ashed and total fluorine in the total body ash determined.

METHOD USED FOR DETERMINATION OF FLUORINE

The fluorine method was originally proposed by Willard and Winter (*9*) and was modified by Armstrong (*10*). The procedure has been further modified by Hoskins and Ferris (*11*). They introduced into the titration a buffer system of sodium hydroxide and chloroacetic acid, in the ratio of 0.5 and a total concentration at 0.02 M. Recently, Armstrong (*12*) announced a microprocedure based on the titration of fluorine in an aqueous solution rather than the usually alcoholic solution. The quantity of perchloric acid apt to be evolved in the distilling process is reduced according to Armstrong (*12*) by distilling the hydrofluosilicic acid in the presence of sodium perchlorate. The procedure for the removal of chloride when present in interfering amounts calls for the use of silver perchlorate. To eliminate any interference of phosphate in the thorium nitrate titration, Churchill, Bridges, and Rowley (*13*) have found it necessary to make a double Willard and Winter distillation using sulfuric acid in the first and perchloric acid in the second distillation. The method is not yet perfected (*14, 15*) but is perhaps one of the more promising which may be applied to microgram quantities of fluorine as occur in biological materials.

Because of the high phosphate content of the materials to be analyzed, the interference of phosphate was studied particularly. The recovery results were not satisfactory. It was found that repeated 200 cc. volumes of distillate from 1 to 5 gm. samples of pure dicalcium phosphate gave small but significant titrations with thorium nitrate. At this time the method of Armstrong for microdeterminations appeared, and a study of his modifications was undertaken. The titration in aqueous solution of pure standard fluoride solutions showed an average variation of about 0.12 gamma of fluorine. Recovery of added fluorine was unsatisfactory. (In all cases we have used the steam distillation procedure as directed by Hoskins and Ferris (*11*).) The titration results were uniformly low. After numerous trials the observed low results were found to be due to evaporation of the slightly alkaline distillate in porcelain containers. Evaporation in glass also caused a slight distortion of results. Platinum containers were adopted therefore for the concentration of the distillate. In the presence of aluminum silicate the thorium nitrate titration in alcoholic solution has been shown to give low results (*15*). It may be suggested that the aqueous microtitration is sensitive to traces of alumina or silica, which hot dilute alkali might dissolve from porcelain. The titration also seems to be more sensitive to the pH of the solution than Armstrong reports. As applied to the alcoholic titration, Carter (*14*) has pointed out that below a pH of 1.2 to 1.4 titrations are considerably higher and above 3.0 to 3.4 there is a marked decrease. Titrations at a pH of 1.6 to 3.0 decrease almost linearly with increase in pH. These same effects may hold for aqueous solutions as employed in the microtitration. Although Armstrong reports a buffer is unnecessary if care is used to adjust the acidity of the solutions before titration, for convenience the use of the buffer is included in Armstrong's directions.

An effect of dicalcium phosphate on the microprocedure also was observed at least for quantities of 0.1 gm. or more of dicalcium phosphate. This is in agreement with the observation of Churchill, Bridges, and Rowley (*13*) as applied to alcoholic titration.

The procedure as carried through in the analysis of the samples of bone, tooth, and body ash, and of the basal rations is as follows: The sample of ash is placed in a 50 cc. Claissen pyrex distilling flask to which is added 0.1 gm. of acid-washed ignited silica, 3 quartz beads, 10 cc. of water, and 5 cc. of 60 percent perchloric acid. Where possible a sample is taken so as to give 50 to 100 gamma of total fluorine. An auxiliary flask containing distilled water is attached in order to pass steam into the distilling flask. Both flasks are heated simultaneously, the temperature in the distilling flask being kept at 135° to 140°. The distillate is kept slightly alkaline to phenolphthalein, by adding a dilute solution of sodium hydroxide. Approximately 150 cc. of distillate are collected, requiring one-half hour to collect. The alkaline distillate is evaporated in a platinum dish on the water bath to about 5 cc. volume, then made just acid with dilute hydrochloric acid, and made to a volume of 10 cc. One-tenth aliquots (1 cc.) are titrated with thorium nitrate having a fluorine equivalent of 4 to 5 gamma per cc. For the titration, cylindrical vials approximately 4.5 by 1.4 cm. are used. One drop of a 0.05 percent aqueous solution of sodium alizarine sulfonate is added, and the color of the indicator brought to the transition point with very dilute hydrochloric acid or sodium hydroxide. One drop of the Hoskins-Ferris buffer is added to the contents, shaken and allowed to stand a few minutes, before titrating with standard thorium nitrate. The end point is recognized as a light pink or orange color. From 4 to 6 aliquots are titrated to obtain an average titration figure.

Reagents and standard thorium nitrate are prepared as directed by Hoskins and Ferris (*11*). The purity of sodium fluoride used to prepare the standard fluoride solutions was determined by conversion to the sulfate and weighing. It was found that the buffer tends to change on standing and should be prepared frequently. The titration values increased with the age of the buffer, indicating perhaps an effect of pH on the titration as has been shown by Carter (*14*) and mentioned above.

In the recovery tests of 10 to 100 gamma of fluorine, added to the distilling flask, the error in the determination as we have found it may equal 1 to 5 gamma (in cc. of thorium nitrate equivalent to 4.5 gamma of fluorine per cc., this variation equals approximately 0.02 to 0.10 cc. in actual titration). Frequently this error was less than 1 gamma of fluorine. In using a sample of ash containing from 50 to 100 gamma of fluorine, the error is reduced to a maximum of about 10 percent with an expected average error of about 5 percent in terms of actual fluorine determined.

Thus far our studies and use of this micromethod proposed by Armstrong (*12*) have not shown quite the accuracy obtained by Armstrong. The method would appear to have particular value insofar as it is possible to use minimum quantities of reagents and samples, thus eliminating, in a degree, interfering elements from these sources. Dahle and Wichmann (*16*) have pointed out the effects of soluble salts of non-volatile acids and volume of liquid in the distilling flask in the actual distilling process. The minimum quantities of materials (sulfuric acid 10 cc., perchloric acid 10 cc., sodium perchlorate 20 gm., monocalcium phosphate 5 gm., ammonium sulfate 15 gm., sodium sulfate 15 gm.) as studied by Dahle and Wickmann must be regarded as excessive in comparison with 5 cc. perchloric acid, 0.1 gm. silica and a 0.01 to 0.10 gm. sample of bone or tooth ash employed in Armstrong's procedure. As is shown by Armstrong in aqueous solution also, the concentration of perchloric acid required to influence the results is greater than in alcoholic solution. In our experience the end point seems slightly more distinct in aqueous solution than in alcoholic.

TABLE 1.—*Fluorine in food and water, daily fluorine intake, food and water intake, initial and final body weight, and average daily gain of experimental rats*

SERIES 1-50 P. P. M. NaF IN DRINKING WATER OF RATS 41, 44, 47

Rat no. and sex	Days on experiment	Average fluorine content of ration	Average fluorine content of water	Average daily F intake per kgm. body weight	Initial body weight	Final body weight	Average body weight	Average daily gain in weight	Average daily food intake	Average daily water intake	Average daily water intake per square meter of body surface
		P. p. m.	*P. p. m.*	*Mg.*	*Gm.*	*Gm.*	*Mg.*	*Gm.*	*Gm.*	*Gm.*	
40♂...	49	4.0	0.0	.31	62.3	179.5	120.9	2.39	9.50	8.82	398
41♂...	49	4.0	22.6	2.11	59.3	167.0	113.1	2.20	9.50	8.88	417
42♂...	49	25.4	0	2.04	61.7	175.5	118.6	2.53	9.50	10.08	460
43♀...	49	4.0	0	.35	57.6	137.0	97.3	1.62	8.36	9.91	516
44♀...	49	4.0	22.6	2.53	59.6	137.5	98.6	1.59	8.34	9.53	452
45♀...	49	25.5	0	2.11	59.3	143.5	101.4	1.72	8.40	10.38	526
46♂...	49	4.4	0	.33	53.0	136.7	94.9	1.71	6.98	7.66	407
47♂...	49	4.5	22.6	2.57	57.0	136.7	96.9	1.63	7.02	9.65	506
48♂...	49	36.6	0	2.47	54.3	154.0	104.2	2.03	7.01	10.31	512

RESULTS OF EXPERIMENT

The data for average daily intake of food, water, and fluorine, initial, final, and average body weight and the daily gain in body weight, are shown in table I. As was mentioned, the individual food intake was equalized for the three rats of a group. The water intake of rats 13 to 27 was not controlled, and a greater water intake by the food-fluoride rats, with the exception of rat 24, is apparent. The tendency to drink more water was less pronounced in the case of the water-fluoride rats, possibly due to their ability to detect the presence of fluoride in the drinking water. In the other series of trials (rats 28 to 51) an attempt was made to restrict water consumption to the same level for all rats of a group, by preventing access to the drinking fountain for several hours during the day. This procedure was only partially successful but enough so that the rats of a group averaged only about 2 cc. difference in daily water intake. In view of the obvious possibilities of variable water intake arising in an experiment of this type, where individual rats may refuse water or consume large quantities of water due to the fluoride factor as well as to individual idiosyncracies, this item of experimental control appears unavoidable.

As indicated by refusal to consume the day's allotment of food, the rats' appetites were adversely affected by fluoride ingestion only in case of fluoride rats of series 4. The food refusals of all the rats are summarized as follows:

Series No.	F in drinking water p. p. m.	Number of times the daily food allotment was not entirely consumed		
		Control rats	Water-fluoride rats	Food-fluoride rats
1	22.6	58	40	12
2	45.2	25	33	20
3	90.4	21	26	12
4	135-226	29	116	226

There is the suggestion in the results for the rats of series 1, that a concentration of fluorine, particularly in the food, equal to 22.6 p.p.m. of fluorine in the drinking water, may have had a stimulating effect on appetite.

Table 2 shows the results of fluorine determinations in the bones and incisor teeth and the total body ash and the calculated total fluorine per kilogram of final empty body weight. In table 3 appears the data covering the balance of fluorine. In table 4 a statistical analysis of the data is summarized. The probability factors are calculated according to "Student's" method (*17, 18*) as applicable to small numbers of differences. The data comprising the 1st, 2d, and 3d series are treated separately as to series for some comparisons but as a single set of data also for all different effects compared. All the fluoride rats, both water- and food-fed fluoride, are combined in each series and compared with their controls. Because the duration of the experiment as well as the method and level of feeding (600 p.p.m. of fluorine in the diet of food-fluoride rats) of series 4, vary quite widely from series 1, 2, and 3, the data for this group are analyzed separately. The data of these tables and other observations may be discussed most advantageously under the following topics:

AVERAGE DAILY BODY GAIN

Growth curves for individual rats appear in graph I. A decided effect on rate of growth resulting from the ingestion of 0.06 percent fluorine in the food is demonstrated here and has been shown by previous work (*19, 20, 21, 22, 23*). The intermittent ingestion of fluoride in the water in variable quantities, ranging from 135-226 p.p.m. of fluorine, lowered the growth rate. During periods of acute toxicity the appetite of water-fluoride rats (Nos. 14, 17, 20, 23, 27) failed seriously with an expected decline in weight, apparent in the growth curves. Fluoride water was withdrawn when a recovery seemed unlikely, and appetite and growth responded accordingly. Our results in this case agree with the fatalities and inhibited growth produced by 226 p.p.m. of F in the drinking water of rats tested

TABLE 1.—*Fluorine in food and water, daily fluorine intake, food and water intake, initial and final body weight, and average daily gain of experimental rats*—Continued

SERIES 2—100 P. P. M. NaF IN DRINKING WATER OF RATS 50, 53, 56

Rat no. and sex	Days on experiment	Average fluorine content of ration	Average fluorine content of water	Average daily F intake per kgm. body weight	Initial body weight	Final body weight	Average body weight	Average daily gain in weight	Average daily food intake	Average daily water intake	Average daily water intake per square meter of body surface
		P. p. m.	*P. p. m.*	*Mg.*	*Gm.*	*Gm.*	*Mg.*	*Gm.*	*Gm.*	*Gm.*	
49♀	49	4.5	0	.37	55.3	138.7	97.0	1.70	8.06	10.39	541
50♀	49	4.5	45.2	5.48	53.0	134.7	93.8	1.67	8.06	10.55	568
51♀	49	62.3	0	5.32	54.3	134.0	94.2	1.62	8.03	10.90	579
52♀	49	4.5	0	.34	54.7	140.7	97.7	1.76	7.42	10.24	532
53♀	49	4.5	45.2	5.32	53.7	136.3	95.0	1.69	7.42	10.42	554
54♀	49	64.7	0	4.92	53.3	139.7	96.5	1.76	7.34	11.02	578
55♂	49	4.5	0	.34	55.0	177.7	116.4	2.50	8.62	9.47	438
56♂	49	4.5	45.2	4.52	53.7	176.3	115.0	2.50	8.62	10.62	493
57♂	49	59.6	0	4.66	53.7	166.3	110.0	2.30	8.61	10.55	505

SERIES 3—200 P. P. M. NaF IN DRINKING WATER OF RATS 29, 32, 35, 38

Rat no. and sex	Days on experiment	Average fluorine content of ration	Average fluorine content of water	Average daily F intake per kgm. body weight	Initial body weight	Final body weight	Average body weight	Average daily gain in weight	Average daily food intake	Average daily water intake	Average daily water intake per square meter of body surface
28♂	49	13.9	0	1.13	57.6	155.3	106.5	2.20	8.15	9.00	442
29♂	49	12.2	90.4	8.53	67.0	135.3	101.2	1.39	8.09	9.36	474
30♂	49	108.2	0	8.15	58.6	159.0	108.8	2.05	8.15	8.86	429
31♀	49	15.6	0	1.22	52.3	161.0	106.7	2.22	8.34	10.23	502
32♀	49	12.5	90.4	7.92	52.7	166.5	106.6	2.32	8.35	8.86	435
33♀	49	101.2	0	7.92	52.7	160.5	106.6	2.20	8.34	9.97	489
34♂	49	15.9	0	1.19	59.0	149.5	109.3	1.85	8.09	9.72	468
35♂	49	13.2	90.4	6.79	58.0	143.0	100.5	1.73	8.07	7.90	403
36♂	49	84.9	0	6.57	61.7	148.5	105.1	1.77	8.12	9.43	465
37♀	49	9.2	0	.76	55.7	129.5	92.6	1.51	7.96	10.51	567
38♀	49	13.7	90.4	9.39	63.3	133.5	98.4	1.43	8.02	9.84	508
39♀	49	114.7	0	10.36	55.3	122.0	88.7	1.36	8.01	10.32	573

SERIES 4—200-700 P. P. M. NaF IN DRINKING WATER OF RATS 14, 17, 20, 23, 26

Rat no. and sex	Days on experiment	Average fluorine content of ration	Average fluorine content of water	Average daily F intake per kgm. body weight	Initial body weight	Final body weight	Average body weight	Average daily gain in weight	Average daily food intake	Average daily water intake	Average daily water intake per square meter of body surface
13♀	70	3.3	0	.19	61.3	126.0	93.7	.92	5.64	9.38	499
14♀	70	3.3	84.4	9.32	63.7	107.7	85.7	.63	5.55	9.25	526
15♀	70	605.9	0	36.48	68.3	118.3	93.3	.71	5.62	10.42	558
16♂	71	3.4	0	.19	89.3	147.7	118.5	.82	6.60	13.33	609
17♂	71	3.5	167.1	14.90	92.3	135.7	114.0	.61	6.49	10.03	469
18♂	71	605.8	0	34.51	89.0	141.3	115.2	.74	6.56	14.24	662
19♂	62	3.5	0	.21	87.0	136.0	111.5	.79	6.42	8.99	425
20♂	62	3.3	126.2	11.05	86.3	129.0	107.7	.69	6.26	9.27	452
21♂	62	606.0	0	36.27	86.0	127.5	106.8	.67	6.39	15.11	737
22♀	70	3.4	0	.23	60.0	116.3	88.2	.80	5.92	14.56	809
23♀	70	3.4	133.3	11.74	64.7	119.3	92.0	.78	5.92	7.95	429
24♀	70	605.8	0	39.53	67.0	113.0	90.0	.66	5.87	14.28	782
25♀	70	3.4	0	.23	54.0	109.0	82.0	.79	5.44	7.39	430
26♀	70	3.4	115.0	13.76	61.3	104.3	82.8	.61	5.39	9.74	562
27♀	70	605.8	0	40.61	57.7	103.3	80.5	.65	5.40	9.78	574

TABLE 2.—*Fluorine content of ash of bodies, bones and teeth; ash and fluorine content of dry fat-free bones and teeth; total fluorine per kilogram of final empty body weight*

SERIES 1—50 P. P. M. NaF IN DRINKING WATER OF RATS 41, 44, 47

Rat No.	Average fluorine content of ration	Fluorine content of drinking water	Average daily fluorine intake per kgm. body wt.	Fluorine content of body ash	Ash content of dry fat-free bones	Fluorine content of bone ash	Fluorine content of dry-fat-free bones	Ash content of dry fat-free incisor teeth	Fluorine content of tooth ash	Fluorine content of dry fat-free incisor teeth	Total fluorine per kgm. of final empty body-weight
	P. p. m.	P. p. m.	Mg.	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Mg.
40	4.0	0.0	0.31	0.012	63.06	0.014	0.009	77.61	0.016	0.013	3.85
41	4.0	22.6	2.11	.087	64.08	.108	.069	77.36	.051	.040	28.90
42	25.4	0	2.04	.088	64.77	.108	.070	77.51	.056	.043	30.72
43	4.0	0	.35	.014	63.47	.017	.011	77.01	.019	.015	5.01
44	4.0	22.6	2.53	.097	63.47	.118	.075	77.73	.057	.044	32.16
45	2[illegible].5	0	2.11	.094	63.38	.123	.078	77.37	.061	.047	33.07
46	4.4	0	.33	.014	60.38	.017	.010	75.97	.019	.015	5.02
47	4.5	22.6	2.57	.113	59.54	.152	.091	76.45	.066	.051	39.49
48	36.6	0	2.47	.118	60.29	.149	.090	76.48	.065	.050	42.09
SERIES 2—100 P. P. M. NaF IN DRINKING WATER OF RATS 50, 53, 56											
49	4.5	0.0	0.37	0.012	61.24	0.015	0.009	75.02	0.020	0.015	4.50
50	4.5	45.2	5.48	.195	62.39	.256	.160	76.67	.106	.081	77.18
51	62.3	0	5.32	.224	62.29	.266	.166	76.34	.106	.081	78.10
52	4.5	0	.34	.013	62.17	.013	.008	76.44	.019	.014	5.16
53	4.5	45.2	5.32	.211	62.37	.258	.161	78.43	.114	.087	81.36
54	64.7	0	4.92	.209	61.86	.261	.161	76.26	.123	.094	84.43
55	4.5	0	.34	.011	60.70	.014	.009	77.99	.013	.013	3.81
56	4.5	45.2	4.52	.170	60.47	.238	.144	77.81	.110	.086	53.99
57	59.6	0	4.66	.197	60.94	.281	.172	77.95	.119	.093	71.39
SERIES 3—200 P. P. M. NaF IN DRINKING WATER OF RATS 29, 32, 38											
28	13.9	0.0	1.13	0.051	63.20	0.064	0.041	77.08	0.019	0.015	16.67
29	12.2	90.4	8.53	.368	64.66	.427	.276	76.44	.203	.155	131.45
30	108.8	0	8.15	.344	64.86	.491	.318	76.89	.199	.153	131.27
31	15.6	0	1.22	.051	60.95	.071	.043	75.76	.041	.031	17.05
32	12.5	90.4	7.92	.376	62.01	.432	.268	76.34	.232	.177	118.26
33	101.2	0	7.92	.358	61.23	.475	.291	75.90	.212	.161	116.05
34	15.9	0	1.19	.054	61.92	.069	.043	76.13	.038	.029	17.36
35	13.2	90.4	6.79	.315	60.86	.388	.236	75.33	.166	.126	109.61
36	84.9	0	6.57	.305	61.64	.381	.235	75.74	.171	.130	110.87
37	9.2	0	.76	.036	62.20	.053	.033	76.56	.030	.023	14.44
38	13.7	90.4	9.39	.378	62.64	.510	.320	76.69	.209	.160	146.64
39	114.7	0	10.36	.350	62.46	.488	.305	76.89	.238	.183	143.94
SERIES 4—200-700 P. P. M. NaF IN DRINKING WATER OF RATS 14, 17, 20, 23, 26											
13	3.3	0.0	0.19	0.0[illegible]5	61.31	0.024	0.015	78.81	0.023	0.018	6.02
14	3.3	84.4	9.32	.614	61.86	.746	.462	77.91	.400	.312	246.16
15	605.9	0	36.48	.956	61.17	1.671	1.020	84.70	1.072	.908	459.56
16	3.4	0	.19	.012	64.97	.022	.014	77.91	.031	.024	5.39
17	3.5	167.1	14.90	.902	65.78	1.040	.684	77.59	.567	.440	382.74
18	605.8	0	34.51	.268	65.60	1.479	.970	78.62	1.081	.850	520.40
19	3.5	0	.21	.012	64.07	.021	.014	75.51	.033	.025	6.01
20	3.3	126.2	11.05	.643	64.99	.777	.505	74.79	.512	.383	262.98
21	606.0	0	36.27	1.262	64.60	1.758	1.136	73.10	1.174	.858	567.48
22	3.4	0	.23	.016	62.65	.024	.015	74.89	.025	.019	6.75
23	3.4	133.3	11.74	.707	63.86	.844	.539	76.20	.509	.388	383.88
24	605.8	0	39.53	1.275	63.74	1.663	1.060	73.91	1.087	.804	[illegible]09.67
25	3.4	0	.23	.015	63.65	.026	.018	76.70	.029	.022	7.05
26	3.4	115.0	13.76	.835	65.03	.953	.620	76.23	.668	.509	349.50
27	605.8	0	40.61	1.422	64.75	1.743	1.129	74.54	1.198	.893	642.12

by Sebrell et al. (*1*) and Dean et al. (*2*).

Combining the growth data of series 2 and 3 for food-fluoride rats versus controls, the probability factor of 0.002 is obtained, indicating with practical certainty that growth was impaired at levels of 45.2 and 90.4 p.p.m. of fluorine in the food. Taking all the fluoride rats in series 2 versus their controls, a probability factor of 0.030 is obtained. A similar factor as obtained from the growth data of series 3 equals 0.070. These factors indicate significant differences in the two sets of data, and these results pertaining to growth may be said to indicate strongly an effect of 45.2 p.p.m. and 90.4 p.p.m. of fluoride in food and water on growth. Fluoride equaling 22.6 p.p.m. of fluorine in drinking water may possibly have retarded growth, but similar quantities in the food seem to have increased growth slightly.

The data for rats of series 4 indicate beyond question that all the fluoride-fed rats gained less than their controls. An adverse effect on growth thus obtains also for 600 p.p.m. of fluorine in the food and 90.4 to 316.6 p.p.m. of fluorine given irregularly in the drinking water.

The work of previous investigators has shown in general that retarded growth in young rats does not result until the fluoride intake equals 200 to 300 p.p.m. of fluorine (*2, 20, 21, 22, 23*). Sollmann, Schettler and Wetzel (*24*) report a level of 90.4 p.p.m. of fluorine as retarding growth under ad libitum feeding.

The relation of water-ingested versus food-ingested sodium fluoride to the average daily gain may best be evaluated by statistical analysis of the data. Considering water-fluoride rats versus food-fluoride rats in series 1, 2, 3, the mean difference equals −0.12 gm. less average daily gain for the water-fluoride rats. The probability factor is 0.082, indicating that this difference could happen by chance once in 11 or 12 trials. From this it cannot be concluded that water-fed fluoride at these 3 levels affected growth more seriously than food-fed fluoride. However, it must be admitted that combining the data for these 3 levels (22.6, 45.2, and 90.4 p.p.m. of fluorine) disregards the possibility that water-fed fluorides versus food-fed fluorides at a level of 22.6 p.p.m. may show differences not observable at 90.4 p.p.m. In fact, the rats fed 22.6 p.p.m. in food versus water showed directly opposite effects on growth compared with their controls. By comparing the three food-fed rats with their comparable water-fed rats, a statistically significant difference is obtained. A slightly lower fluorine intake on the basis of kilogram of body weight for the food-fluoride rats

TABLE 3.—*Total fluorine intake in food and water during experimental period; fluorine in final body weight; and total fluorine retained from intake*

SERIES 1—50 P. P. M. NaF IN DRINKING WATER OF RATS 41, 44, 47

Rat No.	Treatment	Total fluorine intake			Calculated total fluorine in initial body weight	Total fluorine in final body weight	Total fluorine retained from intake	
		In ration	In water	Total				
		Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Percent
40	Control	1.86	0.0	1.86	0.44	0.66	0.22	11.82
41	NaF in water	1.86	9.84	11.70	.42	4.53	4.11	35.13
42	NaF in ration	11.81	0	11.81	.43	5.06	4.63	39.20
43	Control	1.64	0	1.64	.40	.64	.24	14.64
44	NaF in water	1.64	10.56	12.20	.42	4.19	3.77	30.90
45	NaF in ration	10.51	0	10.51	.42	4.48	4.06	38.63
46	Control	1.52	0	1.52	.37	.64	.27	17.76
47	NaF in water	1.53	10.69	12.22	.37	5.05	4.68	38.30
48	NaF in ration	12.58	0	12.58	.38	5.87	5.49	43.64

SERIES 2—100 P. P. M. NaF IN DRINKING WATER OF RATS 50, 53, 56

Rat No.	Treatment	In ration	In water	Total	Initial body weight	Final body weight	Retained	Percent
49	Control	1.77	0.0	1.77	0.39	0.58	0.19	10.73
50	NaF in water	1.77	23.40	25.17	.37	9.74	9.37	37.23
51	NaF in ration	24.55	0	24.55	.38	9.68	9.30	37.88
52	Control	1.62	0	1.62	.38	.67	.29	17.90
53	NaF in water	1.62	23.12	24.74	.38	10.35	9.97	40.30
54	NaF in ration	23.26	0	23.26	.37	11.06	10.69	45.96
55	Control	1.91	0	1.91	.39	.64	.25	13.09
56	NaF in water	1.91	23.56	25.47	.38	8.80	8.42	33.06
57	NaF in ration	25.14	0	25.14	.38	11.07	10.60	42.52

SERIES 3—200 P. P. M. NaF IN DRINKING WATER OF RATS 29, 32, 35, 38

Rat No.	Treatment	In ration	In water	Total	Initial body weight	Final body weight	Retained	Percent
28	Control	5.56	0.0	5.56	0.40	2.51	2.11	37.95
29	NaF in water	4.82	37.47	42.29	.47	17.20	16.73	39.56
30	NaF in ration	43.48	0	43.48	.41	19.85	19.44	44.71
31	Control	6.36	0	6.36	.37	2.56	2.19	34.43
32	NaF in water	5.13	36.24	41.37	.37	18.56	18.19	43.97
33	NaF in ration	41.36	0	41.36	.37	17.67	17.30	41.83
34	Control	6.30	0	6.30	.41	2.43	2.02	32.06
35	NaF in water	5.24	28.19	33.43	.41	14.81	14.40	43.07
36	NaF in ration	33.79	0	33.79	.43	15.29	14.86	43.98
37	Control	3.58	0	3.58	.39	1.71	1.32	36.87
38	NaF in water	5.40	39.87	45.27	.44	18.37	17.93	39.61
39	NaF in ration	45.05	0	45.05	.39	16.16	15.77	35.01

SERIES 4—200-700 P. P. M. NaF IN DRINKING WATER OF RATS 14, 17, 20, 23, 26

Rat No.	Treatment	In ration	In water	Total	Initial body weight	Final body weight	Retained	Percent
13	Control	1.34	0.0	1.34	0.43	0.72	0.29	21.64
14	NaF in water	1.32	54.61	55.93	.45	24.89	24.44	43.70
15	NaF in ration	238.28	0	238.28	.48	49.77	49.29	20.69
16	Control	1.59	0	1.59	.63	.75	.12	7.54
17	NaF in water	1.61	119.02	120.63	.65	45.68	45.03	37.33
18	NaF in ration	282.22	0	282.22	.62	68.11	67.49	23.91
19	Control	1.35	0	1.35	.61	.77	.16	11.85
20	NaF in water	1.31	72.47	73.78	.60	31.60	31.00	42.01
21	NaF in ration	240.19	0	240.19	.60	66.31	65.71	27.36
22	Control	1.41	0	1.41	.42	.74	.32	22.70
23	NaF in water	1.40	74.20	75.60	.45	31.67	31.22	41.30
24	NaF in ration	249.06	0	249.06	.47	57.26	57.79	23.20
25	Control	1.29	0	1.29	.38	.73	.35	27.13
26	NaF in water	1.32	78.41	79.73	.43	35.02	34.59	43.38
27	NaF in ration	228.83	0	228.83	.40	60.61	60.21	26.31

may possibly explain their slight advantage in growth over the water-fluoride rats. It remains to be said that the combined evidence is indicative of no variable effects on growth due to food versus water ingestion at levels of 22.6, 45.2, and 90.4 p.p.m. fluorine.

COMPOSITION OF BONES

From the data of table 2 it is apparent that there was a consistent and a highly significant increase in the bone ash of the water-fluoride rats over the control rats (series 4). The difference is less consistent but nevertheless significant for the food-fluoride rats over the controls. In series 1, 2, and 3 the water fluoride rats exceed their controls by +0.32 in percent ash in bone; the food-fluoride rats of these series average +0.44 percent more bone ash than their controls. The probabilities in these comparisons indicate that these values have more than border-line significance. The data on the whole warrant the conclusion that an increase in bone ash, due to fluoride ingestion, is demonstrated. These results confirm previous findings of McClure and Mitchell (*25*) and Chaneles (*26*). They are not in agreement with the results of Kick et al. (*19*) who found little or no change in ash, nor with the results of Smith and Lantz (*27*) and Tolle and Maynard (*28*) who found a decrease in ash content. Hauck, Steenbock, and Parsons (*29*), on the addition of 0.15 percent sodium fluoride found a variable effect on ash content of bone. On a low calcium ration and on the stock diet of moderate calcium content, bone ash was decreased absolutely and proportionately. On a high calcium rachitogenic diet bone ash was definitely increased.

There is no very certain evidence that the percent ash of bones varied with respect to path of fluoride ingestion, for rats in series 1, 2, and 3. We have studied the data for series 1, 2, and 3 separately, as regards differences in food-fed fluoride versus water-fed fluoride on ash of bone, and find no differences to obtain for any one level of intake. The water-fluoride rats showed consistently more ash than food-fluoride rats in series 4. It should be noted here also that food-fluoride rats (Nos. 15, 18, 21, 24) received almost three times as much total fluoride as did their corresponding water-fluoride rats, and thus the level of fluorine intake may possibly explain differences noted.

The data give certain important information relative to the accumulation of fluorine in the bones, the fluorine quantities deposited being definitely correlated with the fluorine intake. As regards the comparison of effects of water-

TABLE 4.—*Summary of statistical analysis of data*

Rats compared	F intake of test rats per kg. body weight	Comparisons			Differences		Probability of chance outcome
		Number			Mean	Standard deviation of mean [1]	
	Mg.	Total	Plus	Minus			
AVERAGE DAILY GAIN—GRAMS DAILY							
Water-F vs. food-F,[2] series 1, 2, 3	2.0–10.4	10	4.0	6.0	−0.12	0.079	0.082
Water-F vs. food-F, series 4	9.3–40.6	5	3.0	2.0	+.02	.023	.368
Water-F vs. controls,[3] series 1, 2, 3	2.0–10.4	10	[4] 2.5	7.5	−.03	.041	.350
Water-F vs. controls, series 4	9.3–14.9	5	0	5.0	−.16	.019	>.001
Food-F vs. controls, series 1, 2, 3	2.0–10.4	10	3.5	6.5	−.01	.053	.464
Food-F vs. controls, series 4	34.5–40.6	5	0	5.0	−.15	.022	>.001
Food-F vs. controls, series 1	2.0–2.5	3	3.0	0	+.19	.067	.034
Food-F vs. controls,[5] series 2, 3	4.5–10.4	7	6.5	.5	−.10	.023	.002
F-rats vs. controls,[5] series 1	2.0–2.6	6	3.0	3.0	+.04	.232	.436
F-rats vs. controls, series 2	4.5–5.5	6	1.0	5.0	−.06	.026	.030
F-rats vs. controls, series 3	6.6–10.4	8	2.0	6.0	−.16	.097	.070
ASH CONTENT OF BONES—PERCENT							
Water-F vs. food-F, series 1, 2, 3	2.0–10.4	10	5.0	5.0	−0.12	0.054	0.024
Water-F vs. food-F, series 4	9.3–40.6	5	5.0	0	+.33	.010	.011
Water-F vs. controls, series 1, 2, 3	2.0–10.4	10	6.5	3.5	+.32	.270	.132
Water-F vs. controls, series 4	9.3–14.9	5	5.0	0	+.97	.146	>.001
Food-F vs. controls, series 1, 2, 3	2.0–10.4	10	7.0	3.0	+.44	.223	.037
Food-F vs. controls, series 4	34.5–40.6	5	4.0	1.0	+.64	.227	.020
F-rats vs. controls, series 1	2.0–2.6	6	3.5	2.5	+.28	.370	.236
F-rats vs. controls, series 2	4.5–5.5	6	4.0	2.0	+.37	.260	.100
F-rats vs. controls, series 3	6.6–10.4	6	6.0	2.0	+.48	.329	.092
ASH CONTENT OF INCISOR TEETH—PERCENT							
Water-F vs. food-F, series 1, 2, 3	2.0–10.4	10	4.0	6.0	−0.01	0.100	0.500
Water-F vs. food-F, series 4	9.3–40.6	5	3.0	2.0	−.41	1.676	.406
Water-F vs. controls, series 1, 2, 3	2.0–10.4	10	5.0	5.0	+.17	.228	.239
Water-F vs. controls, series 4	9.3–14.9	5	1.0	4.0	−.22	.395	.304
Food-F vs. controls, series 1, 2, 3	2.0–10.4	10	5.0	5.0	+.19	.153	.139
Food-F vs. controls, series 4	34.5–40.6	5	2.0	3.0	+.21	1.523	.451
F-rats vs. controls, series 1	2.0–2.6	6	4.0	2.0	+.29	0.154	.055
F-rats vs. controls, series 2	4.5–5.5	6	2.0	4.0	+.43	.338	.126
F-rats vs. controls, series 3	6.6–10.4	8	4.0	4.0	−.14	.161	.205
FLUORINE IN BONES, TEETH AND BODY ASH—PERCENT							
Water vs. food-bones,[6] series 1, 2, 3	2.0–10.4	10	3.5	6.5	−0.009	0.005	0.070
Water vs. food-teeth, series 1, 2, 3	2.0–10.4	10	3.5	6.5	−.003	.007	.357
Water vs. food-body ash, series, 1, 2, 3	2.0–10.4	10	3.5	6.5	−.004	.003	.108
FLUORINE RETAINED FROM TOTAL INGESTED—PERCENT							
Water vs. food-F, series 1, 2, 3	2.0–10.4	10	2.0	8.0	−3.42	1.110	0.006

[1] Standard deviation of mean $=\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

Σd^2=sum of squared deviations from the mean

n=number of comparisons.

[2] Water-F vs. food-F=the water-fluoride rat is plus or minus with respect to its corresponding food-fluoride rat.

[3] Food-F vs. controls=the food-fluoride rat is plus or minus with respect to its corresponding control rat.

[4] Where both rats show the same result a value of 0.5 is credited to each rat.

[5] F-rats vs. controls=the fluoride-fed rat is plus or minus with respect to its corresponding control rat.

[6] Water vs. food=the water-fluoride rat is plus or minus with respect to its corresponding food-fluoride rat.

borne fluoride versus food-borne fluoride, the evidence is not conclusive. The food-fed fluoride (series 1, 2, and 3), if anything, seems to have caused a deposition of slightly more fluorine in the bones than water-fed fluoride. The odds are 14 to 1 against this difference being a chance happening. The results for fluorine in total body ash, which are essentially an estimate of bone-ash composition, give but slight evidence of more fluorine deposited, as due to food-fluoride.

COMPOSITION OF INCISOR TEETH

The data in general reveal no effect of fluoride, as ingested on the ash content of the rat's incisor. There is the suggestion that levels corresponding to 22.6 p.p.m. of fluorine in the drinking water increase the ash slightly. Of the six fluoride-rats fed at this level either in food or water, four showed a slightly greater incisor ash than their controls. The odds are 1 to 18 against this being a fortuitous happening. Food-ingested fluoride versus water-ingested fluoride played no part in determining the values for incisor ash.

Kick et al. (*19*) reported, for rats and swine, chemical analyses of the teeth as not revealing "any striking differences in their organic composition aside from the increase in percentage of fluorine." Smith and Lantz (*27*) and Hauck, Steenbock, and Parsons (*29*) report a decrease in incisor ash irrespective of the calcium level of the diet. Chaneles (*26*) reports a 4-percent decrease in ash of teeth of rats fed sodium fluoride.

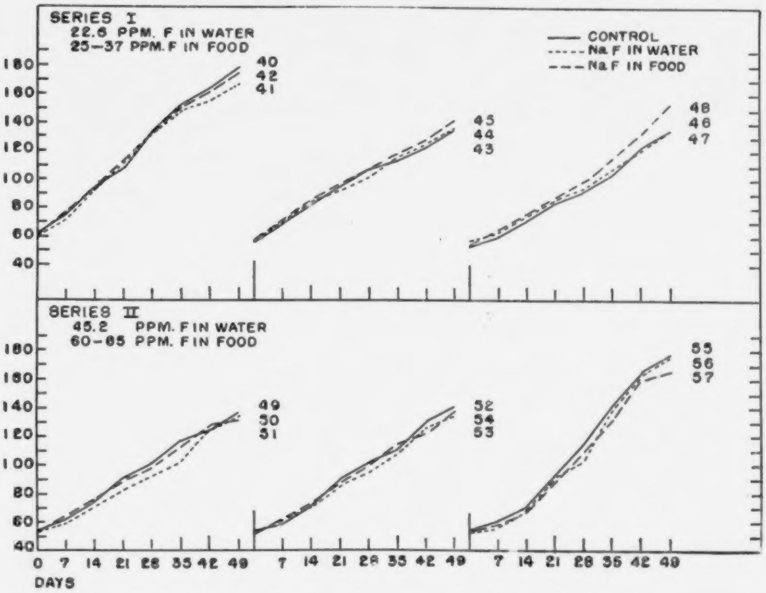
The fluorine content of the teeth, as in the case of the bones, varies directly with the level of intake. There is no evidence that more fluorine appears in the teeth of the water-fluoride rats than in the teeth of food-fluoride rats—series 1, 2, and 3.

Table 5 summarizes the data to show the correlation which exists between the daily fluoride ingested, either in food or water, and the corresponding fluorine content of bones, teeth, and total final empty body weight. This marked fluorine increase in bones and teeth which closely parallels the level of intake has been demonstrated previously in growing rabbits (*30*), rats (*19, 31, 32, 33, 34, 35*), swine (*19*), dairy cows (*36*), and dogs (*37, 38*). Recently Roholm (*22*) in his extensive studies of human fluorosis among cryolite workers, by post-mortem examination demonstrated that there is an accumulation of fluorine in bone tissue accompanying human fluorosis. It is apparent from the data for fluorine present in our rats' bones and teeth, that the bones are the more active depositors and tend to approach a maximum fluorine content more rapidly than do the teeth. These differences in total fluorine retained may be due in part to a more active tissue or mechanism for interchange with circulating fluids on the part of bone tissue. On the basis of different chemical compounds present in bones as compared with dentine and enamel (*39*), qualitative differences in the fluorine complexes present in the two types of tissues also may be surmised.

PHOTOGRAPHIC STUDIES OF INCISOR TEETH

The enlarged photographic studies (figs. 1–9) of the lower incisors, provide a delicate measure of the quantity of fluoride ingested. The very fine, closely spaced, brown striations may appear when the fluoride intake approaches from

Graph I. (a.)—*Growth curves for rats of series 1 and 2.*



9 to 15 p.p.m. in food (table 5). Above these levels the striations become progressively more pronounced until they tend to become irregular patches at about 150 p.p.m. or more of fluorine in the intake depending on the duration of the experiment. A chalky white appearance or lightening of the tooth pigment is not apparent to the naked eye at intakes equaling 9 to 15 p.p.m. of fluorine. In photographs of normal teeth there are faint indications of striations, and for the low levels of fluoride-ingestion the photographs in several instances show apparently normal teeth. These inconsistent responses may be due to individual susceptibilities of the animals and are somewhat indicative of the limitations of photographic studies for showing low fluorine concentrations. A large number of individuals would be necessary to establish a more exact correlation between the intake level, duration of ingestion, and the striated appearance. Dean et al. (*2*) reported the appearance of fine striations at levels as low as 10.5 p.p.m. of added fluorine, and obtained indications later of striations at levels of 8 to 10 p.p.m. Smith and Leverton (*3*) observed pigment changes at a level of 14 p.p.m. Ellis and Maynard (*34*) noted but slight changes in pigment at levels of 12 to 16 p.p.m. in the intake.

We are unable to detect any consistent or significant differences in the photographic studies between the teeth from water-fed fluoride rats and comparable food-fed fluoride rats.

The Balance of Fluorine

The total fluorine retained in the final empty body weight, table 3, includes a small fraction of fluorine present in the initial body weight. This average figure as obtained by analysis of six stock rats of average initial body weight, equaled 0.02 percent fluorine, and represents a total of about 0.4 to 0.6 mg. of fluorine in the initial body weight. For the fluoride-fed rats on the lowest levels of intake this represents less than 10 percent of the total fluorine in their final weight. In the case of control rats whose final body weight contained a total of as low as 0.58 to 0.60 mg. of fluorine per kilogram, upwards of 60 to 70 percent of final total fluorine is represented as initial fluorine. Such small residue figures, representing accumulation from the intake, but due practically entirely to a growth increment, are not significant as balance data.

The data for control rats of series 3 (rats 28, 31, 34, and 37) when compared with similar data for other control rats, shows these rats as having ingested more fluorine, and having a corresponding greater quantity of fluorine in the final empty body weight. The food-fluoride intake of all the rats of this series was unintentionally increased on the seventh day of the fourth week, by a mistake in ration allotment. Whereas the average daily intake of fluorine for controls, water-fluoride rats and food-fluoride rats in these groups equaled approximately, 0.04 mg., 1.0 mg., and 1.0 mg., respectively, daily throughout the experiment, on this one day the total food-fluorine ingested averaged about 4.5 mg. for controls, 3.5 to 4.0 mg. for water-fluoride rats and 3.0 to 4.0 mg. for the food-fluoride rats. Thus all three rats of a group ingested practically identical quantities of food fluoride on this day. The quantity of fluoride ingested also is far from toxic in itself. Since the outcome of a controlled experiment of this type is based on comparative results, obtained from control versus test rats, the significance of the final comparative data was not impaired by this day's increased fluoride intake, the quantity being the same for all three rats of a group.

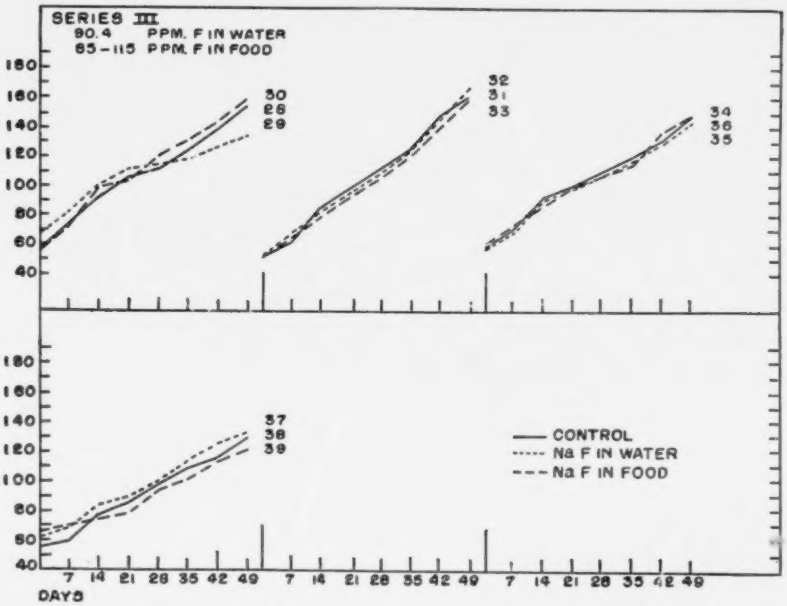
Table 5.—*Summary table showing relation of level of fluorine intake to fluorine content of bones, teeth, and final empty body weight*

Number of rats represented	Days on experiment	Fluorine content of ration	Fluorine content of drinking water	Daily fluorine ingested per kg. body weight	Fluorine content of dry fat-free bones	Fluorine content of dry fat-free incisor teeth	Fluorine per kg. final empty body weight	Appearance of teeth
		P. p. m.	*P. p. m.*	*Mg.*	*Percent*	*Percent*	*Mg.*	
5	63	3.3- 3.5	0.0	0.19- 0.23	0.014-0.018	0.018-0.025	5.39- 7.05	Normal.
6	49	4.0- 4.5	0	.31- .37	.009- .011	.013- .015	3.81- 5.16	Normal—figs. 1, 4, 7, 10, 13, 16.
4	49	9.2- 15.9	0	.76- 1.22	.033- .043	.015- .051	14.44- 17.36	Normal to striated—figs. 19, 22, 25.
6	49	(1) 25.4- 36.6	(2) 22 6	2.04- 2.57	.069- .091	.040- .051	28.90- 42.09	Striated +++—figs. 2, 3, 5, 6, 8, 9.
6	49	(1) 59.6- 62.3	(2) 45.2	4.52- 5.48	.130- .172	.081- .094	53.99- 84.43	Striated +++++—figs. 11, 12, 14, 15, 17 18.
8	49	(1) 84.9-108.8	(2) 90.4	6.57-10.36	.235- .320	.126- .183	109.61-146.64	Striated ++++++—figs. 20, 21, 23, 24, 26, 27.
5	63	3.3- 3.5	135.0-226.0	9.32-14.90	.462- .684	.312- .509	246. 2-382.74	Irregular patches.
5	63	605.8-606.0	0	34.51-40.61	.970-1.136	.804- .908	459. 6-642.12	Do.

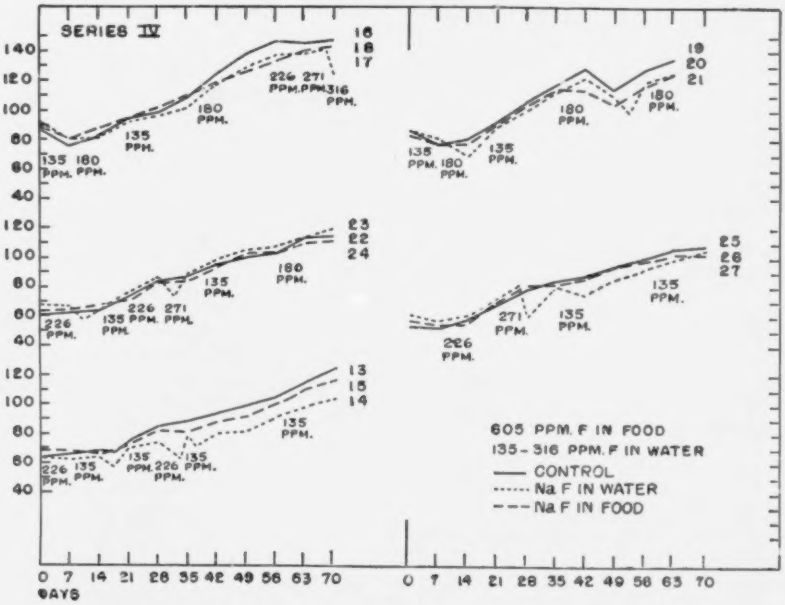
[1] Water contained 0.0 p. p. m. F when these rations were fed.

[2] Rations contained 4.0-12.5 p. p. m. F when these waters were drunk.

Graph I. (b.)—*Growth curves for rats of series 3.*



Graph I. (c.)—*Growth curves for rats of series 4.*



In series 1, 2, and 3 the average percent retention equals about 40 percent of the intake for all fluoride-fed rats, and is about the same for the water-fluoride rats of series 4. The retention figures are quite consistent among these rats whose daily intake, however, ranges from 2 to 15 mg. per kilogram average body weight. The food-fluoride rats of series 4 which received from 34.5 to 40.6 mg. of fluorine daily per kilogram body weight retained from 20.7 to 27.4 percent of the total fluorine ingested.

In 8 out of 10 possible comparisons, the water-fluoride rats retained less total fluorine than the food-fluoride rats. The average difference equals −3.42 percent. The standard deviation is 1.110, and the probability 0.006. This latter figure indicates that this difference would occur but once in 166 trials. This is a significant statistical result, and the data may be said to indicate a slightly greater retention from the food-fed fluoride than from equal quantities of sodium fluoride ingested in the drinking water. Since the growth data for these rats as a group cannot be shown to indicate a significantly greater average daily gain, the retention figures represent a slightly greater total concentration of fluorine in bodies of food-fluoride rats. This result is in agreement with the observed tendency of the water-fluoride rats to show a slight decrease of fluorine in bones and body ash as compared with food-fluoride rats.

The previous recorded results pertaining to the retention of fluorine from sodium fluoride are somewhat limited. In 1891 Brandl and Tappeiner (*37*) fed variable daily quantities of sodium fluoride to a young growing dog for about 21 months. From a total of 402.9 gm. fed, 72.6 gm., or approximately 18 percent of the intake, were deposited in the body. Kick et al. (*19*), fed (in a group) three young male stock rats, weighing 125 gm. each, for a period of 18 days sodium fluoride equal to about 117 mg. fluorine daily per kilogram body weight. Approximately 32.5 percent was retained.

Phillips, Hart, and Bohstedt (*40*) added sodium fluoride to milk diets of young rats equivalent to 0.5, 1.0, and 2.0 p.p.m. of fluorine, and on analysis of the final body ash found an increased residual body fluorine. The percent fluorine stored varied widely among the groups analyzed, i.e., from 20.7 to 76.5 percent for rats fed milk mineralized with sodium fluoride. The quantities of fluorine present in the rats' weight at the start of the fluoride feeding was not estimated by these investigators; and the quantities of fluorine ingested were extremely small, varying from 4.40 to 18.48 mg. of total fluorine for a 140-day feeding period. DuToit, Smuts, and Malan (*33*) studied, by direct metabolism tests, the balance of fluorine ingested as sodium fluoride. From a daily intake of 3.15 mg. to 3.43 mg. of fluorine, the percent fluorine retained by four rats varied from 32.9 to 46.2 percent. These retention figures approximate our data for 35 to 40 mg. fluorine intake per kilogram body weight, and probably represent a maximum of absorbing capacity at levels of about 40 mg. per kilogram body weight. Somewhat below these levels (2 to 10 mg. per kg. daily) during a period of rapid growth, the rat retains, as our data show, as much as 40 to 45 percent of the total fluorine ingested.

Some data relative to the retention of fluorine from other fluorine compounds are available in the experiments of Kick et al. (*19*) and DuToit et al. (*33*). According to Kick et al., rats weighing 125 gm. fed fluorine, as in rock phosphate and sodium fluosilicate, retain about 30 percent of the ingested fluorine when fed at a level of about 10 to 20 mg. fluorine daily. Fluorine fed as calcium fluoride in a similar experiment showed less than 0.1 percent retention. On the other hand, DuToit and his coworkers fed 738.4 mg. fluorine "in the form of $CaHPO_4$ and CaF_2" to two bovines and found 34.05 and 31.6 percent fluorine retained. The retention from 60.6 mg. of ingested fluorine from the same source equaled 12.9 percent and 18.1 percent, according to DuToit et al. It appears scarcely probable, however, that, as stated by DuToit et al., "the percentage retention of fluorine is increased with increased fluorine feeding."

The inhalation of hydrogen fluoride by experimental animals also has been shown to increase to a marked degree the total fluorine in bones and teeth (*30*), the quantities found being dependent on hours of exposure and concentration of hydrogen fluoride in the air.

The fluorine content of the bones and teeth and the total fluorine in the final empty body weight have been plotted against the fluorine intake in milligrams per kilogram body weight daily for rats of series 1, 2, and 3 (graph II). The graph needs little explaining. The close correlation between fluorine intake and total quantities deposited is obvious. The percent retention decreases at higher intake levels (series 4), but is possibly at its maximum under conditions of maximum growth and low intake level, as represented by the data reproduced in the graph.

Our results pertaining to chronic fluorosis and the comparisons we have made thus far with regard to food-ingested fluoride versus water-ingested fluoride may be summarized briefly as follows:

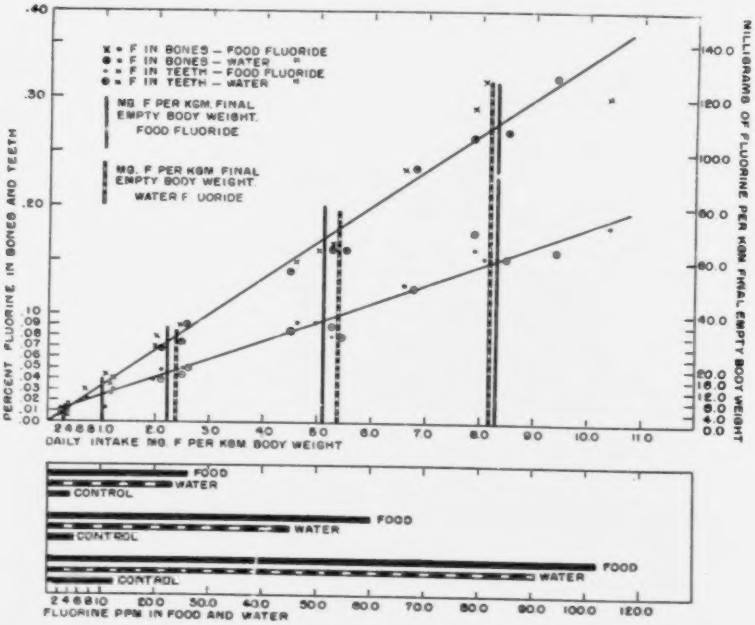
(*a*) There was the suggestion of a stimulating effect of 22.6 p.p.m. of fluorine in the diet on appetite and daily body gain.

(*b*) The stimulating effect of ingested fluorides on water-drinking was again observed.

(*c*) Fluorine levels of 45.2 p.p.m. or more in water and food gave evidence of a reduced rate of gain, but no variations in body gain due to food versus water ingestion occurred at levels of 45.2 to 90.4 p.p.m.

(*d*) An increase in ash content of bones due to fluoride ingestion at levels of sodium fluoride equaling 22.6 to 600 p.p.m. of fluorine is demonstrated, but no differences relate to the mode of ingestion.

Graph II.—*Retention of fluorine in bones and teeth and in final empty body weight after seven weeks period of ingestion of sodium fluoride via food or water.*



(*e*) Fluorides accumulated in the bones and the teeth of experimental rats at a rather remarkable rate—the average total retention of fluorine from the intake equaled 30 to 40 percent. The water-fluoride rats retained slightly less total fluorine than did the food-fluoride rats.

(*f*) Photographic studies of the teeth show that enamel striations appear at about 15 p.p.m. of fluorine in the intake. The quantity of fluorine in the teeth is directly proportional to the observed defects.

(*g*) In general it may be said that insofar as an effect may be shown by daily body gain, ash and fluorine content of bones and teeth, the appearance of the teeth and the total fluorine retained, there were no differences to indicate that water ingestion of sodium fluoride at levels corresponding to 22.6, 45.2, and 90.4 p.p.m. of fluorine in water was more detrimental than food ingestion of similar quantities of sodium fluorides.

CONCLUSIONS

With growing young rats as the experimental animal, chronic fluorosis was studied particularly from the standpoint of severity of effects produced by sodium fluoride—in quantities equivalent to 22.6 p.p.m., 45.2 p.p.m., and 90.4 p.p.m. of fluorine in the drinking water—ingested in the food, as compared with similar quantities ingested in the drinking water. So far as may be shown by (*a*) the average daily body gain, (*b*) ash and fluorine content of bones and teeth, (*c*) the appearance of the teeth, and (*d*) the total fluorine retained in the final body weight, there were no noticeable differences which could be attributed to either of the two mediums of fluoride ingestion.

Acute toxic effects of sodium fluoride ingested in the drinking water at a level of about 180 p.p.m. of fluorine were observed, thus confirming previous observations made at the National Institute of Health. These acute effects of water-ingested fluorides at certain high levels of intake are not to be confused with conditions and effects accompanying fluoride consumption in drinking waters of mottled enamel areas.

Judging from fluorine balance studies on growing young rats, the average total retention of fluorine from sodium fluoride may equal 30 to 40 percent of the intake, at the concentrations studied. By far the greater portion of this fluorine is deposited in the bones and teeth.

The degree of tooth hypoplasia in the rat is directly correlated with the quantity of fluorine present. It has been suggested that the fluorine content of the bones and teeth of experimental animals may be developed into a biologic test for effective fluorides in foods as well as a means of indicating a pathologic condition in the affected tissue.

Observations on the effects of sodium fluoride as fed to young rats include the following: (*a*) there is the suggestion of a stimulating effect of 22.6 p.p.m. of fluorine in the diet, on appetite and perhaps on the daily body gain; (*b*) the stimulating effects of fluorides on water-drinking was again observed; (*c*) sodium fluoride at a level equivalent to 45 p.p.m. or more of fluorine in food and water gave evidence of a reduced rate of gain; (*d*) an increase in ash content of bones occurred from sodium fluoride ingested at levels equaling 22.6 to 600 p.p.m. of fluorine.

In the case of the rat, 0.03 to 0.04 percent fluorine represents approximately the maximum that may be present in the whole tooth and the tooth enamel still retain its normal macroscopic appearance.

Although it is extremely difficult to judge the actual conditions of ingestion, and while positive statements are not warranted, it would appear than an accumulation of fluorine in the bones of adults living in areas where mottled enamel is endemic may be expected. An accumulation of abnormal and perhaps pathologic quantities of fluorine in the bones of adults living in these areas appears within the realm of probability.

Future study of chronic fluorosis requires that emphasis be placed on the localization of fluorine in the organism particularly in the bones and teeth. There is a need for more information relative to quantities of fluorides in foods, the toxicity of natural food fluorides as compared with pure inorganic fluorides, the associative factors involved in fluoride absorption and metabolism, and direct information is needed from epidemiological biochemical studies relative to the involvement particularly of bone tissue among the human populations of mottled enamel areas.

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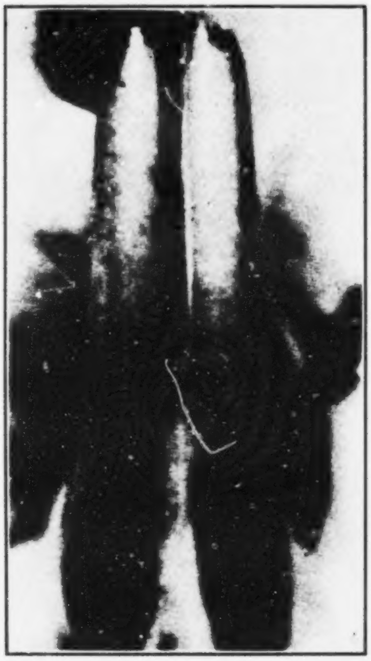

Figure 1.—*Lower incisors of control rat 40. Daily F intake 0.31 mg. per kgm. body weight; 0.013 percent F in teeth; 0.009 percent F in bones.*

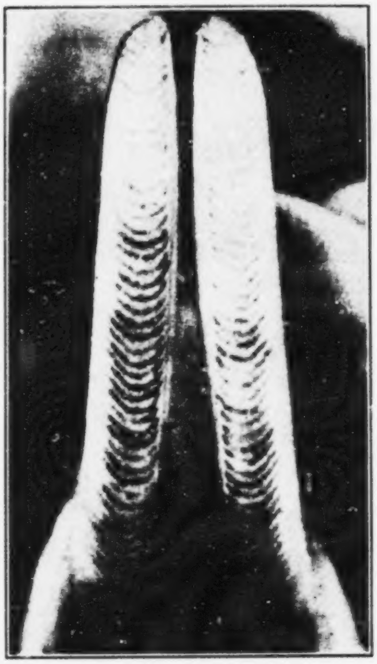

Figure 2.—*Lower incisors of water-fluoride rat 53. Daily F intake 5.32 mg. per kgm. body weight; 0.087 percent F in teeth; 0.161 percent F in bones.*

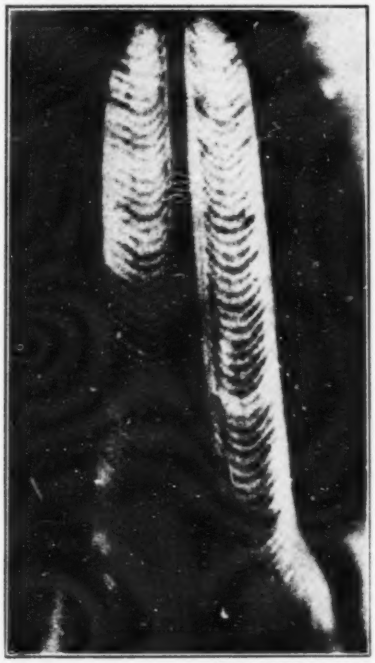

Figure 3.—*Lower incisors of food-fluoride rat 54. Daily F intake 4.92 mg. per kgm. body weight; 0.094 percent F in teeth; 0.161 percent F in bones.*

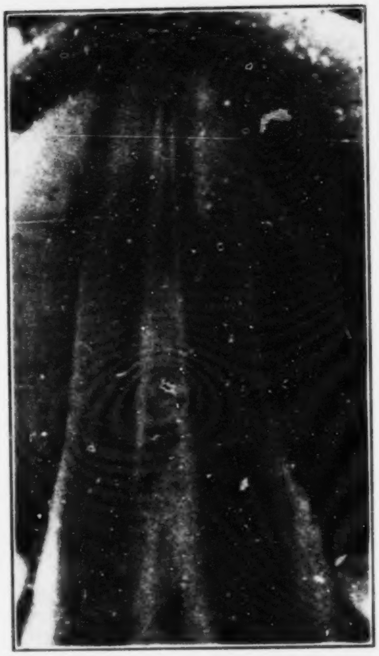

Figure 4.—*Lower incisors of control rat 31. Daily F intake 1.22 mg. per kgm. body weight; 0.029 percent F in teeth; 0.043 percent F in bones.*

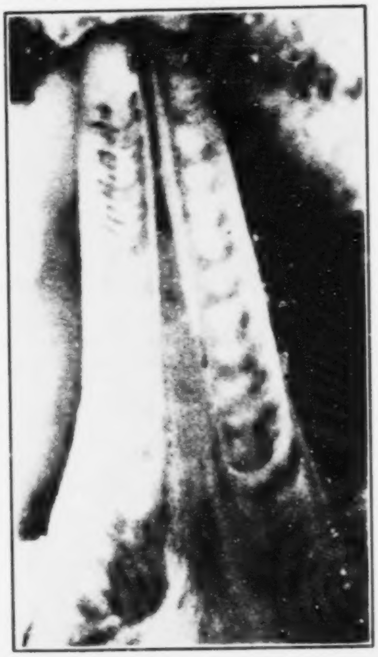

Figure 5.—*Lower incisors of water-fluoride rat 32. Daily F intake 7.92 mg. per kgm. body weight; 0.177 percent F in teeth; 0.268 percent F in bones.*

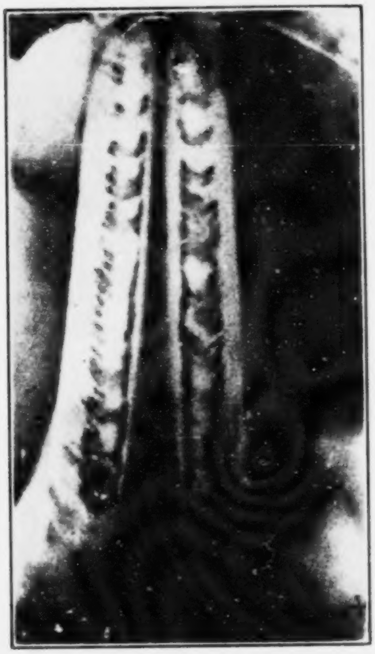

Figure 6.—*Lower incisors of food-fluoride rat 33. Daily F intake 7.92 mg. per kgm. body weight; 0.161 percent F in teeth; 0.291 percent F in bones.*

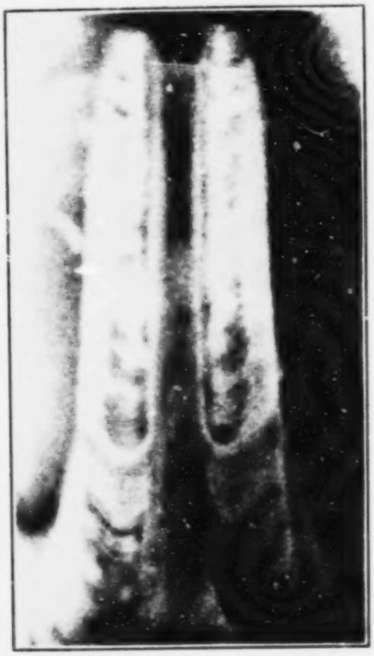

Figure 7.—*Lower incisors of water-fluoride rat 35. Daily F intake 6.79 mg. per kgm. body weight; 0.126 percent F in teeth; 0.236 percent F in bones.*

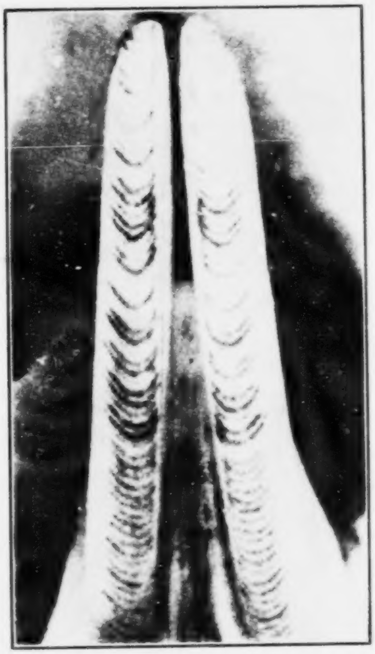

Figure 8.—*Lower incisors of food-fluoride rat 57. Daily F intake 4.66 mg. per kgm. body weight; 0.093 percent F in teeth: 0.172 percent F in bones.*

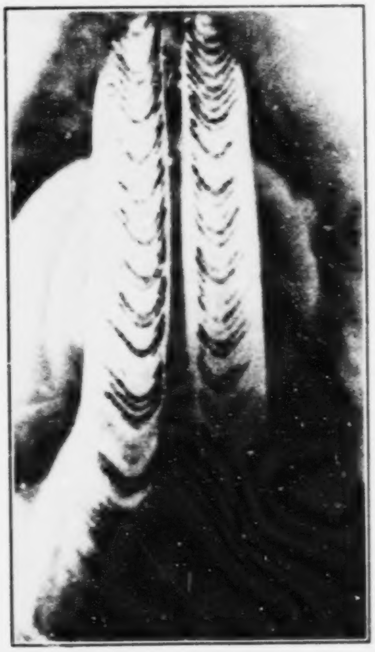

Figure 9.—*Lower incisors of food-fluoride rat 39. Daily F intake 10.36 mg. per kgm. body weight; 0.183 percent F in teeth; 0.305 percent F in bones.*

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FLUORINE ACQUIRED BY MATURE DOG'S TEETH

F. J. McClure

The post-eruptive deposition of fluorine in the enamel (*1, 2, 3*) and dentine (*2, 3*) of molar teeth of young rats has been reported. A similar study on a mature dog supports this finding particularly with regard to the dentine. At the beginning of this study a two-year-old mongrel dog with full dentition had three representative teeth extracted. Sodium fluoride was then given via food and drinking water for definite periods, each period terminating with the extraction of two or three comparable teeth. Food and drinking water were consumed ad libitum. The pooled teeth representing each period of exposure to fluoride were separated into dentine and enamel and analyzed for ash and fluorine.

The results for each successive sample of dentine show that the mature dentine increased in fluorine decisively. The enamel does not show a similarly consistent nor equal percentage-increase. The final sample of enamel, however, contained 0.011 percent fluorine as compared with 0.006 percent fluorine in the initial or control enamel. In this connection it is interesting to note that the enamel of carious human teeth and the enamel of non-carious human teeth have been reported to contain 0.0069 percent and 0.0111 percent fluorine, respectively (*4*). A distinction possibly may be made in tooth fluoride acquired posteruptively and fluoride which is acquired during the formative period of the tooth. (*2, 3*). Fluoride given during gestation and lactation to mother rats, i.e., during the period of tooth formation in their young, appears to diminish susceptibility to caries in the offspring (*5*). There is certain limited epidemiological evidence with respect to human dental caries, (*6*) which seems to agree with this latter finding in rats (*5*).

These results for the dog's teeth and similar results based on young rats advance speculation regarding post-eruptive chemical modification, as a property of calcified dentine and enamel. Fluoride retention may prove a useful tool in studying individual variations in dentine and enamel in relation to tooth age and susceptibility to dental caries. In the results for the enamel fluoride the property of the enamel to absorb fluoride on the oral surface may receive further support, although a systemic retention via the dentine can not be discounted.

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Table I

Effect of Exposure to Fluoride on the Fluorine Content of the Dentine and Enamel of a Mature Dog's Teeth

Pooled teeth sample number		I	II	III	IV	V
Age of dog at time teeth were extracted.	(days)	730	796	900	1,145	1,289
Total number of days exposed to fluoride in food and water prior to tooth extraction	(days)	0	66	170	415	559
Exposure to fluoride immediately prior to tooth extraction.						
Fluoride in food and water	(ppm)	0	15	45	100	500
Total time fed	(days)	0	66	104	245	144
Composition of dentine						
Ash	(per cent.)	71.90	71.88	73.15	73.35	73.21
Fluorine	(per cent.)	.018	.022	.039	.059	.072
Composition of enamel						
Ash	(per cent.)	95.20	95.75	95.72	96.32	95.96
Fluorine	(per cent.)	.006	.007	.009	.007	.011

BLOOD HEMOGLOBIN AND HEMATOCRIT RESULTS ON RATS INGESTING SODIUM FLUORIDE

F. J. McClure and Arthur Kornberg

A reduction of blood hemoglobin in rats receiving 50 p.p.m. of fluorine as sodium fluoride in their drinking water was observed recently in studies by Ginn and Volker (*1*). On the 30th, 44th, 65th and 86th days of fluorine exposure in that experiment, the average hemoglobin results for 18 rats were respectively as follows: 15.2, 14.7, 11.6 and 11.1 grams per 100 cc. Control rats maintained a level of 15.8 grams of hemoglobin per 100 cc. In a previous study with rabbits, Valjavec (*2*) injected a 1.0 percent solution of sodium fluoride intravenously daily over a period of 105 to 159 days so as to provide 10 to 30 mgm. fluorine per kgm. body weight daily. There appeared to be only a slight reduction in hemoglobin and red cell counts in these animals. Greenwood, Hewitt and Nelson (*3*), however, found no change in the hemoglobin and blood coagulation time resulting from sodium fluoride fed in milk to young dogs. Quantities of fluorine varied from 0.45 to 4.52 mgm. per kgm. body weight daily, determinations on the blood being made every two weeks over a period of 18 weeks.

Blood examinations in human cases exposed to the fluorine-mineral cryolite, were made by Roholm (*4*). Among 66 workers in cryolite factories, Roholm observed a slight reduction in the number of erythrocytes but hemoglobin values were unaffected. There were minor variations from the normal in the data for differential counts. Coagulation time was tested also, all such data being considered "within the normal boundaries" (*4*). In the majority of these workers some osteosclerosis due to fluorine was reported, indicating a relatively excessive fluorine exposure.

The uncertainties surrounding the relation of fluoride ingestion to blood hemoglobin and particularly the recent hemoglobin results of Ginn and Volker (*1*) suggested the accumulation of the data as reported in tables I and II. Six studies of control and test rats included three strains of this species, four diets, several different periods of exposure and two levels of fluorine exposure. (See table I.) With the exception of Experiment 6 these blood studies were one phase of other experiments planned to study fluorosis or rat dental caries. In Experiment 6 the rats received a Hoppert-Webber-Canniff diet (*5*) as modified by Ginn and Volker (*1*), i.e. Comet brand brown rice replaced the coarse corn meal in that diet. The composition of the diets in all six experiments is shown in table III. With the exception of Experiment 4 (table I), fluorine exposure consisted of 50 p.p.m. fluorine as sodium fluoride in the drinking water. The excessive fluorine content of diet 530 (Exp. 4) was due to 1.0 percent rock phosphate which contained approximately 3.6 percent fluorine.

Blood determinations were made on specified dates in Experiments 5 and 6 (see table II) and at the termination of Experiments 1, 2, 3, and 4 (table I). Hemoglobin was determined by the method of Evelyn (*6*). Hematocrit values were obtained according to Van Allen (*7*).

Inspection of the hemoglobin and hematocrit values in tables I and II reveals no relation of these data to the fluoride exposures. It may be noted that 50 p.p.m. fluorine in rats' drinking water is sufficient to cause a very noticeable bleaching or striated appearance in rats' incisors and frequently causes excessive incisor-elongation. In general, this fluor-

Table I. — *Hemoglobin and hematocrit determinations for rats given fluoride in food or drinking water*

	Experiment											
	1		2		3		4		5		6	
	Strain of rats											
	N.I.H.		Duke U.		Sherman		N.I.H.		N.I.H.		N.I.H.	
Number of rats	7	15	5	10	8	5	10	12	18	18	23	23
Days on experiment (days)	120	120	365	365	365	365	135	135	86	86	86	86
Fluorine added to water (p.p.m.)	—	50[1]	—	50	—	50	—	—	—	50	—	50
Fluorine added to food (p.p.m.)	—	—	—	—	—	—	—	360	—	—	—	—
Diet no.	550	550	550	550	550	550	531	530	510	510	516	516
Iron in diet (p.p.m.)	118	118	118	118	118	118	9	170	15	15	35	35
Hemoglobin (gm. per 100 ml.)	15.1	15.4	15.0	14.7	14.8	14.4	15.0	15.1	14.5	14.9	13.2	13.0
Hematocrit (volume—percent)	46.1	44.9	44.0	44.7	44.3	45.6	44.5	44.4	33.6	33.7	40.0	40.4

[1] During the last 60 days, 100 p.p.m. of fluorine was added to the drinking water.

Table II. — *Periodic hemoglobin and hematocrit determinations for control rats vs. rats receiving 50 p.p.m. fluorine in drinking water*

Blood Analysis	Experiment 5[1]					Experiment 6[2]				
	No. of rats	Hemoglobin[3]		Hematocrit[4]		No. of rats	Hemoglobin		Hematocrit	
		Control	Fluoride	Control	Fluoride		Control	Fluoride	Control	Fluoride
1st(30th day)	18	12.1	12.0	38.4	38.9	24	10.9	11.1	39.6	39.8
2d (44th day)	18	—	—	—	—	24	13.8	13.7[1]	42.3	41.5
3d (65th day)	18	13.2	12.2	42.8	42.3	22	13.5	14.6	40.7	42.1
4th(86thday)	18	14.5	14.9	33.6	33.7	23	13.2	13.0	40.0	40.4

[1] Diet 510 was fed in this experiment.
[2] Diet 516 was fed in this experiment.
[3] Grams per 100 cc. blood.
[4] Volume percent.

TABLE III.—*Percentage composition of diets*

	Diet No.				
	550[1]	530	531	510	516
Corn meal	—	—	—	69.0	—
Corn starch	68.5	—	64.0	—	—
Corn flour	—	62.5	—	—	—
Brown rice (Comet brand)	—	—	—	—	60.0
Whole milk powder	25.0	30.0	30.0	30.0	30.0
Yeast	5.0	3.0	3.0	—	—
Salt	0.5	1.0	1.0	1.0	1.0
Cod liver oil	1.0	2.0	2.0	—	—
Linseed meal	—	—	—	—	5.0
Alfalfa meal	—	—	—	—	3.0
$CaCO_3$	—	0.5	—	—	—
Rock-phosphate	0	1.0	—	—	—

[1] Had added 100 p.p.m. Fe and 10 p.p.m. Cu.

ine exposure in rats is regarded as corresponding roughly to 5 p.p.m. fluorine in domestic water supplies, a fluorine exposure to which human population groups may be subjected (*8*).

While a fluorine anemia may occur as in Ginn and Volker's study, this effect presumably is associated with unspecified or unknown factors not duplicated in our studies. The possibility that a marginal nutritional iron deficiency would influence these results was to be considered. Diet 510, in Experiment 5, however, contained only 15 p.p.m. of iron and no detrimental effects of fluorine appear in the data for this experiment. In Experiments 2 and 3, it may be noted also, the rats were on experiment as long as 1 year. It is doubtful that a fluorine anemia should be anticipated under a variety of conditions such as were employed in our experiments.

SUMMARY

The relation of fluorine exposure to blood hemoglobin and hematocrit values in rats was studied in several strains of the species, using several diets and for different experimental periods. There were no differences between control and test animals to indicate any effect of fluorine on hemoglobin and hematocrit values for these rats' blood. It does not appear, according to these results, that 50 p.p.m. fluorine in the drinking water affects the hemoglobin concentration and total red cell volume in the blood of this species.

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INHIBITORY EFFECT OF FLUORIDE ON TOOTH DECALCIFICATION BY CITRATE AND LACTATE IN VIVO

I. ZIPKIN and F. J. MCCLURE

INTRODUCTION

Within recent years, decalcification of molar teeth in vivo has been produced experimentally in a number of laboratory animals (*1–7*). Extensive loss of molar dentin and enamel in vivo was first reported in rats given dilute acids and acid drinks and beverages in place of drinking water (*1*). Similar results have been noted in hamsters, monkeys, and puppies (*2, 3, 4*). The low pH of these acid solutions in most cases accounted for the observed tooth destruction.

The presence of citrate in many soft drinks and fruit juices (*8*) suggested in addition a specific decalcifying effect of the citrate ion, in keeping with its known solubilizing effect on insoluble calcium salts (*9–12*). As was anticipated, therefore, citrate solutions caused a significant decalcification of rats' molar tooth surfaces, not duplicated by lactate solutions at the same nearly neutral pH (*6*).

Recently, Wynn and Haldi (*5*) observed the decalcifying effects of a number of fruit and vegetable juices on rats' molar tooth surfaces in vivo, but were not able to correlate consistently the pH or the amount of citrate presumed to be present in the fluids with the amount of tooth destruction. Their results seemed to lend additional support to the suggestion that other anions in addition to citrate may decalcify tooth surfaces in vivo (*6, 13, 14*).

The role of fluorine in inhibiting tooth decalcification in vivo has been explored thus far most extensively by Restarski, Gortner, and McCay (*2*) Gortner, Restarski, Bieri, and McCay (*3*), and Bieri, McCay, Restarski, and Gortner (*4*). Their results, in general, demonstrate a significant inhibitory effect of fluoride on the tooth decalcifying action of phosphoric, sulfuric, lactic, and citric acids, and of sucrose-phosphoric acid mixtures.

As little as 1.0 p.p.m. fluorine appeared to be inhibitory, but the degree of protection afforded did not seem to vary directly with the amount of fluoride incorporated in the test solutions (*3*).

It was the purpose of this study, therefore, to obtain additional evidence regarding the effect of fluoride on acid decalcification produced particularly by lactic acid, and to ascertain also the effect of fluoride on nonacid decalcification brought about by the citrate ion. Citrate solutions of pH 6.3 to 6.5 were used in this study. Although these solutions are slightly acidic, the term "nonacid" refers to the effect of the citrate anion, since the hydrogen ion is practically ineffective in producing loss of tooth substance at this pH. The effect of intraperitoneally injected fluoride on acid and nonacid decalcification was also studied in an attempt to evaluate the significance of the oral surface effects of fluoride. The general plan of the study is indicated in tables I and II. Weanling rats of approximately 21 days of age were divided into tetrads composed of litter mates. The study was divided into experiments A, B, C, and D, and each experiment consisted of a preliminary period of 28 days and a test period of variable length. Before giving the tooth substance-removing fluids, the respective fluoride solutions alone were offered ad libitum during the 28-day preliminary period in an attempt to "fluorinate" the teeth. The Control and "F Inject" groups received distilled water during this period, and the F Inject groups were injected intraperitoneally with 0.5 mg. fluorine daily (1.0 ml. of a sodium fluoride solution containing 500 p.p.m. fluorine).

Following the preliminary period, the test solutions containing added fluoride were administered as the sole source of fluid, as indicated in tables I and II. The Control and F Inject groups received the test solutions without added fluoride, and the F Inject groups received 0.5 mg. of fluorine per day injected intraperitoneally. The diet, consumed ad libitum, was made up as follows: cornstarch, 66.5 percent; whole milk powder, 27 percent; yeast, 5 percent; cod liver oil, 1 percent, and sodium chloride (containing added traces of iron and copper), 0.5 percent. This diet was adequate for normal growth in rats.

At the end of the test period, the rats were sacrificed and the heads were autoclaved to facilitate removal of the jaws for examination. The molar teeth were scored according to the method proposed by Restarski, Gortner, and McCay (*15*). The average score for the teeth of each quadrant per rat is presented in tables I and II.

The data were submitted to statistical analysis. Inasmuch as experiments A and B are essentially similar, the data were

Table I.—*Inhibition by fluoride of the destructive action of citrate on rats' molar teeth*

EXPERIMENT	A CITRATE-FLUORIDE I				B CITRATE-FLUORIDE II			
Drinking fluid	Citrate	Citrate + 5 ppm F	Citrate + 50 ppm F	Citrate	Citrate	Citrate + 1 ppm F	Citrate + 2.5 ppm F	Citrate + 5 ppm F
Concentration of drinking fluid	0.25 N	0.25 N	0.25 N	0.25 N	0.25 N	0.25 N	0.25 N	0.25 N
pH of drinking fluid	6.3-6.5	6.3-6.5	6.3-6.5	6.3-6.5	6.3-6.5	6.3-6.5	6.3-6.5	6.3-6.5
Number of rats	11	10	11	10	7	8	14	11
Group designation	Control	5 ppm	50 ppm	F Inject	Control	1 ppm	2.5 ppm	5 ppm
Days of experiment	98	98	99	98	98	97	95	92
Fluid intake per rat (ml.)	2000	1991	1992	1979	1807	1811	1815	1817
Fluorine received during test period (mg.)	0.00	9.96	99.6	49.0	0.00	1.81	4.54	9.08
Scoring of Extent of "Erosion" *Average score per rat*								
Upper right molars	5.06	2.43	3.21	4.30	4.48	4.21	3.89	2.45
Upper left molars	4.73	2.53	2.73	4.44	4.67	3.83	3.59	2.41
Lower right molars	5.15	3.17	2.61	4.60	5.19	4.21	3.76	2.55
Lower left molars	4.97	2.37	2.61	4.57	5.24	4.38	4.10	3.09
Upper molars	4.89	2.48	2.97	4.37	4.57	4.02	3.75	2.43
Lower molars	5.06	2.77	2.61	4.58	5.22	4.29	3.93	2.82
All molars	4.98	2.62	2.79	4.48	4.89	4.16	3.84	2.63

Table II.—*Inhibition by fluoride of the destructive effect of lactate on rats' molar teeth*

EXPERIMENT	C LACTATE-FLUORIDE I				D LACTATE-FLUORIDE II			
Drinking fluid	Lactate	Lactate + 5 ppm F	Lactate + 50 ppm F	Lactate	Lactate	Lactate + 1 ppm F	Lactate + 2.5 ppm F	Lactate + 5 ppm F
Concentration of drinking fluid	0.25 N	0.25 N	0.25 N	0.25 N	0.25 N	0.25 N	0.25 N	0.25 N
pH of drinking fluid	3.0-3.2	3.0-3.2	3.0-3.2	3.0-3.2	4.5	4.5	4.5	4.5
Number of rats	11	10	12	8	18	17	18	17
Group designation	Control	5 ppm	50 ppm	F Inject	Control	1 ppm	2.5 ppm	5 ppm
Days on experiment	69	74	68	70	27	29	29	29
Fluid intake per rat (ml.)	739	720	733	747	560	568	568	568
Fluorine received during test period (mg.)	0.00	3.60	36.7	35.0	0.00	0.57	1.42	2.84
Scoring of Extent of "Erosion" *Average score per rat*								
Upper right molars	4.15	2.80	2.90	5.42	2.26	2.33	1.04	1.28
Upper left molars	4.45	2.97	2.78	5.27	2.11	1.43	1.19	1.20
Lower right molars	Destruction too extensive to measure				4.83	4.80	4.65	4.47
Lower left molars					4.78	4.67	4.08	4.82
Upper molars	4.31	2.84	2.82	5.35	2.17	1.88	1.11	1.24
Lower molars	--	--	--	--	4.83	4.73	4.34	4.64
All molars	--	--	--	--	3.50	3.31	2.73	2.94

combined and submitted to analysis of variance (*16*) as shown in table III. In this treatment, the data for the 50 p.p.m. and the F Inject groups were omitted. The former data were not included since it is apparent that 50 p.p.m. fluorine affords no further protection against erosion than 5 p.p.m. fluorine. The data for the F Inject group were omitted since they represent systemically acquired fluorine as compared to orally administered fluorine. The data of Experiment C were analyzed by applying Fisher's "t" test, to determine the significance of the difference between the means. The data of experiment D were studied by means of the analysis of variance, and the results are expressed in table IV.

RESULTS

It is apparent from experiment A, that 50 p.p.m. fluorine afforded no more protection against erosion by citrate at pH 6.3 to 6.5 than did 5 p.p.m. fluorine. Another study, experiment B, was instituted, therefore, to determine the minimum concentration of fluoride which would produce maximum protection against citrate (nonacid) erosion. It was found that increasing protection against citrate erosion occurred in the order of 1, 2.5, and 5.0 p.p.m. fluorine. The erosion from solutions containing 5 p.p.m. fluorine in experiment B was essentially the same as that observed from 5 p.p.m. fluoride solutions in experiment A, thus indicating that 5 p.p.m. fluorine may be the minimum concentration affording maximum protection against erosion produced by citrate.

Analysis of the data indicates that there is a significant difference in the effect of 1 p.p.m., 2.5 p.p.m., and 5 p.p.m. fluorine in inhibiting this type of erosion; that the inhibition is related to the dosage of fluoride as indicated by the significance of the regression line, and that the regression line adequately describes the data, i.e., the individual values adhere closely to the regression line and do not deviate from it significantly.

Application of Fisher's "t" test indicates that there is no significant difference in the erosion score between rats receiving 5 p.p.m. and 50 p.p.m. fluorine. It is noteworthy, however, that fluorine injected intraperitoneally appears to cause a significant reduction in erosion when compared to the Control, that is, the probability of occurrence of the observed difference between the means by chance is less than 0.01.

To determine the effect of fluoride in inhibiting acid erosion at a low pH, lactic acid at pH 3.0 to 3.2 was administered to rats. Erosion was very pronounced and destruction of the lower molars was so severe that scoring was impossible by the usual method. The upper molars, however, were scored as indicated in experiment C, table II. The average score per rat for experiment C is recapitulated below:

Group	*Mean*	*No. of Rats*
Control	4.31 ± 0.398	11
5 ppm	2.84 ± 0.437	10
50 ppm	2.82 ± 0.480	12
F Inject	5.35 ± 0.232	8
		41

It will be noted that again 5 p.p.m. fluorine afforded maximum protection, but that injected fluoride in this experiment was entirely ineffective against an acid erosion. It is difficult to explain the apparent potentiating effect of injected fluoride on erosion produced by lactate as indicated by comparing the means of the Control and F Inject groups by Fisher's "t" test. The probability of occurrence by chance of the observed difference between the means is between 0.02 and 0.05.

Inasmuch as lactic acid at pH 3.0 to 3.2 produced an erosion in the lower molars too severe to measure, experiment D was planned to investigate the destructive effect of lactic acid at pH 4.5, and the ability of 1, 2.5, and 5 p.p.m. fluorine to inhibit its action. The results are shown in Table II, and analysis of the data is expressed in table IV. In view of the pronounced difference of the effect of lactate solutions at pH 4.5 on the upper and lower

TABLE III.—*Statistical analysis of combined data of citrate-fluoride experiments A and B*

GROUP DESIGNATION	AVERAGE SCORE PER RAT	NO. OF RATS
1. Control	4.95 ± 0.129*	18
2. 1 ppm	4.16 ± 0.208	8
3. 2.5 ppm	3.80 ± 0.331	14
4. 5 ppm	2.62 ± 0.213	21
5. 50 ppm	2.79 ± 0.267	11
6. Inject	4.48 ± 0.078	10
		82

Analysis of Variance
(Groups 1, 2, 3, and 4)

COMPONENT OF VARIANCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE	PROBABILITY
Between treatments	3	18.05	22.23	< 0.01
Regression	1	53.44	65.81	< 0.01
Deviations from regression	2	0.355	0.437	> 0.05
Within treatments	57	0.812		

*Deviations from the mean are expressed as the standard error.

TABLE IV
ANALYSIS OF THE DATA OF EXPERIMENT D

GROUP	UPPER TEETH MEAN	NUMBER OF ANIMALS	LOWER TEETH MEAN
Control	2.17 ± 0.290	18	4.83 ± 0.059
1 ppm	1.88 ± 0.282	17	4.73 ± 0.107
2.5 ppm	1.11 ± 0.180	18	4.34 ± 0.141
5 ppm	1.24 ± 0.128	17	4.64 ± 0.090
		70	

Analysis of Variance
Upper Teeth

COMPONENT OF VARIANCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE	PROBABILITY
Between treatments	3	44.83	5.194	< 0.01
Regression	1	9.68	10.419	< 0.01
Deviations from regression	2	2.41	2.591	> 0.05
Within treatments	66	0.93		

Lower Teeth

COMPONENT OF VARIANCE	DEGREES OF FREEDOM	MEAN SQUARE	F VALUE	PROBABILITY
Between treatments	3	0.800	4.470	< 0.01
Regression	1	0.518	2.894	> 0.05
Deviations from regression	2	0.941	5.257	< 0.01
Within treatments	66	0.179		

molars, the respective data were considered separately and were examined by the analysis of variance.

It appears that the response of the lactate-eroded molars to fluoride differs markedly from the response of citrate-eroded molars. In the latter case, both upper and lower molar teeth show the same degree of inhibition by fluoride. (See table I.)

In the former case, however, fluoride affects the upper and lower molars differently. Analysis of the data shows that inhibition of erosion in the upper teeth by fluoride is related to its concentration as indicated by the significant slope of the regression line. Since the deviations from the regression line are not significant, a straight line adequately fits the data.

In the case of the lower teeth, however, the slope of the regression line is not significant, indicating that the degree of inhibition is not proportional to the fluoride concentration. Moreover, the deviations from the regression line are significant, indicating that a straight line does not fit the data, and that there is a "leveling-off" at 2.5 p.p.m. fluorine.

Table V.—*The effect of administration of fluoride on the fluorine content of incisor and molar dentin and enamel*

EXPERIMENT A					
GROUP	MG. F CONSUMED	INCISORS		MOLARS	
		DENTIN	ENAMEL	DENTIN	ENAMEL
Control	0.00	0.0026	0.0022	0.0052	0.0015
5 ppm	9.96	0.0261	0.0107	0.0269	0.0075
F Inject	49.1	0.0703	0.0193	0.0854	0.0078
50 ppm	99.6	0.2000	0.0694	0.1665	0.0407

Discussion

The results of these experiments are in good agreement with previous observations (*2, 3, 4*) in showing, particularly, that the rat erosion-inhibitory property of fluorine is operative within a relatively narrow range and at a very low concentration of fluoride (1 to 5 p.p.m. fluorine). In experiment D, table II, 2.5 p.p.m. fluorine was as effective as 5 p.p.m. in the lower teeth, and in experiments A and C, 50 p.p.m. fluorine was no more effective than 5 p.p.m. fluorine. This latter result applied to both lactate (acid) and citrate (nonacid) solutions.

It is known from previous studies in this laboratory that both injected fluoride (*17*) and orally ingested fluoride (*18*) add fluorine posteruptively to rats' molar dentin and enamel. The amount incorporated is proportional to the quantity of fluorine being ingested in the drinking water. Samples of molar dentin and enamel of the rats of experiment A were analyzed for fluorine in this study and the results confirm this posteruptive addition of fluorine. Owing to the very small quantities of enamel available for analysis, the results were accepted with reservation. They indicate, however, that there was approximately 5 times more fluorine in both the molar dentin and enamel of the rats receiving 50 p.p.m. fluorine than in the rats receiving 5 p.p.m. fluorine, as shown in table V.

When fluoride was injected intraperitoneally, increased amounts were found in the incisor and molar dentin and enamel. It should be noted, however, that the increase of fluorine in the enamel was much less than in the dentin. This was particularly true in the case of molar enamel where only a slight increase in the fluorine content was observed as compared to the 5 p.p.m. group, although the former received five times more fluoride. This finding has previously been reported by Arnold and McClure (*17*).

Although more fluorine was incorporated in the enamel of the 50 p.p.m. group than in the 5 p.p.m. group of experiment A, the degree of rat erosion was essentially the same in both groups. It seems probable, therefore, that the quantity of fluorine in the dentin and enamel above that contributed by 5 p.p.m. fluorine in the drinking water has no further preventive influence on rat erosion.

It is of interest to note that the inhibitory effect of fluorine on enamel erosion in rats is operative at much lower concentrations than is the inhibitory effect of fluorine on induced rat caries. In the latter case, fluoride became partially inhibitory at 10 p.p.m. fluorine, was increasingly effective at 50 p.p.m., and was only fully caries-inhibitory at 100 and 125 p.p.m. fluorine (*18*). With respect to the quantitative relations, our rat-erosion results suggest some similarity to the inhibitory effect of fluoride on human dental caries. Thus, Dean (*19*) has pointed out that a marked reduction in human dental caries occurs at 1.0 to 1.5 p.p.m. fluorine in the drinking water, but further increases in water-fluorine concentration seemingly afford little further caries reduction.

More pertinent to our erosion studies, and illustrative also of the absence of a strictly quantitative relation between fluorine and its effect on enamel, are recent observations by Muhler and Van Huysen (*20*). Within the limits of their studies based on measurements of weight loss of powdered enamel, it was observed that a 4.0 percent sodium fluoride solution gave essentially no more protection against enamel decalcification in vitro than did a 0.2 percent solution.

It seems highly probable that the inhibitory effect of fluoride on acid erosion in rats is due to a decrease in enamel solubility, but it is noteworthy also that the antisolubility effect of fluorine in vivo seems to have a limit of effectiveness, which is about 5 p.p.m. of orally ingested fluorine. Further study is needed to explain the effect of fluorine on nonacid citrate erosion.

It is difficult to explain the anomalous effect of lactate on rats' molar teeth. This observation has previously been made by Gortner, Restarski, Bieri, and McCay (*3*) and by McClure and Ruzicka (*6*).

In conclusion, it should be noted that injected fluoride also appeared to have an inhibitory effect on erosion caused by citrate as shown by analysis of the data of table III using Fisher's "t" test. The inhibitory effect of the injected fluoride (49.1 mg.), however, is produced also by only 1 p.p.m. of orally administered fluoride (1.81 mg.).

Injected fluoride had no effect in reducing the erosion produced by lactate solutions as shown in experiment C, table II. Further studies are contemplated to study the effect of injected fluoride in inhibiting acid (lactate) as well as nonacid (citrate) erosion.

SUMMARY

1. In rats' molar teeth, 2.5 p.p.m. fluorine gave maximum protection against erosion by lactate at pH 4.5, and 5 p.p.m. fluorine afforded maximum protection against erosion by citrate at pH 6.3 to 6.5. As little as 1 p.p.m. fluorine is effective in significantly reducing the erosion produced by lactate at pH 4.5 and by citrate at 6.3 to 6.5. A minimum concentration of fluoride exists, beyond which further increases in administered fluorine afford no further protection against dental erosion in rats.

2. Fluorine acquired by intraperitoneal injection appeared to be effective in reducing the erosion produced by citrate. However, more than 25 times more fluorine was necessary to give the same effect produced by 1 p.p.m. fluorine orally administered.

3. Fluorine injected intraperitoneally was ineffective in reducing the amount of erosion produced by lactate.

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AVAILABILITY OF FLUORINE IN SODIUM FLUORIDE VS. SODIUM FLUOSILICATE

By F. J. McClure

Sodium fluoride is the source of fluorine for the majority of current studies testing the efficacy of fluoridation of community drinking water for the partial control of human dental caries (*1, 2, 3, 4, 5*). On the basis of solubility and availability of fluorine, as well as the innocuous character of the accompanying sodium, sodium fluoride is a logical source of fluorine. At the same time any large-scale program of community water fluoridation must calculate its cost, and other fluorine compounds may be available at less expense and may be equally efficacious. Thus, with sodium fluosilicate currently selling at about half the price of sodium fluoride, the cost of chemicals for the fluoridation of 1 million gallons of water at an optimum level of 1.0 p.p.m. fluorine is approximately $2.15 using sodium fluoride and 76 cents for an equivalent quantity of sodium fluosilicate. The market price of these chemicals, according to Chemical and Engineering News for April 24, 1950, is sodium fluoride 95 percent c. l., wks. 0.10½ c. per pound.; sodium fluosilicate, bbl., cl., 0.04½ c. per pound. The latter compound is also known as sodium silicofluoride.

The consideration of a fluosilicate as a fluorine carrier instead of sodium fluoride has raised the question as to the relative physiological effects of a complex chemical such as sodium fluosilicate vs. sodium fluoride.

Generally speaking, the physiological effects of different fluoride salts will depend on their absorbability from the intestinal tract which may be a direct function of solubility, particularly if relatively large quantities are ingested. But if the quantities of fluoride ingested are very small, the effects of differences in solubility will tend to diminish if not to disappear entirely. Such is the situation on the addition of sodium fluoride or sodium fluosilicate to a drinking water where the concentration does not exceed 1.0 p.p.m. fluorine. It is not to be anticipated therefore that the wide difference in solubility between these two compounds would influence their physiological effects when ingested in very dilute solutions. NaF at 18° C. is approximately 4.0 percent soluble whereas Na_2SiF_6 is soluble to the extent of about 0.6 percent.

In dilute aqueous solutions the hydrolysis of these two fluorine salts yielding fluorine ions is comparatively simple in the case of sodium fluoride, which is practically completely ionized, but quite complex and somewhat obscure in the case of sodium fluosilicate. Although several mechanisms have been proposed, the following reactions of sodium fluosilicate account for the presence of fluoride ions in dilute solutions of sodium fluosilicate.

$$\mathrm{Na_2SiF_6} \longrightarrow 2\ \mathrm{Na^+} + \mathrm{SiF_6^{=}}$$
$$2\ \mathrm{H_2O} + \mathrm{SiF_6^{=}} \longrightarrow 4\ \mathrm{H^+} + 6\mathrm{F^-} + \mathrm{SiO_2}$$

The later reaction is postulated by Kubelka and Prištoupil (*6*) and also by Ryss and Slutskaya (*7*). Ryss (*8*) studied the hydrolysis of $\mathrm{Na_2SiF_6}$ at 11° C. at concentrations of 0.02 M. to 0.00048M., and determined the following equilibrium constants:

$$\mathrm{SiF_6^{=}} \longrightarrow \mathrm{SiF_4} + 2\mathrm{F^-}$$
$$K_1 = 0.65 \times 10^{-6}$$
$$\mathrm{SiF_4} + 2\ \mathrm{H_2O} \longrightarrow \mathrm{SiO_2} + -4\ \mathrm{HF}$$
$$K_2 = 1.04 \times 10^{-8}$$
$$\mathrm{SiF_6^{=}} + 2\ \mathrm{H_2O} \longrightarrow \mathrm{SiO_2} + 4\ \mathrm{H^+} + 6\ \mathrm{F^-}$$
$$K_3 = 5.4 \times 10^{-27}$$

Rees and Hudleston (*9*) suggest a slow reaction as follows:

$$\mathrm{SiF_6^{=}} \dashrightarrow \mathrm{SiF_4} + 2\mathrm{F^-}$$

followed by a relatively rapid reaction

$$\mathrm{SiF_4} + 3\ \mathrm{H_2O} \longrightarrow 4\mathrm{HF} + \mathrm{H_2SiO_3}$$

As would be expected in the light of the above evidence, aqueous solutions of sodium fluosilicate have an acid reaction in the range of pH 3.5-4.0, and the hydrolysis is sufficient also to permit a quantitative titration of fluosilicate solutions with a standard alkali or with a standard solution of sodium carbonate.

The physiological effects of sodium fluosilicate vs. sodium fluoride have been observed most often in chronic fluorine toxicosis manifested by the characteristic effects of fluorine on rats' incisor teeth. De Eds and Thomas (*10*) thus compared NaF, $\mathrm{Na_2SiF_6}$ and $\mathrm{Na_3AlF_6}$ at levels of 14-16 p.p.m. fluorine and concluded that chronic fluorine intoxication in rats as manifested by bleaching of the rats' incisors occurred to practically the same extent from all the above fluorides. Smyth and Smyth (*11*) compared barium fluosilicate and cryolite ($\mathrm{Na_3AlF_6}$) when fed to rats at levels of approximately 40 and 80 p.p.m. fluorine and concluded that "the chronic toxicity of these compounds is substantially the same as that of sodium fluoride for the same fluorine content" (*11*). They observed a total of 246 rats and based their conclusions on rate of growth, fecundity, mortality, tooth development, organ pathology, and disease. Smith and Leverton (*12*) studied the effects of a number of different fluorides when fed to rats so as to provide 14 p.p.m. fluorine in the ration. Their conclusions, although based on comparatively few rats, were summarized as follows:

"Fluorine combined as ammonium and potassium fluoride or as barium, potassium and sodium fluosilicate was found to have the same acute toxicity as when combined as sodium fluoride . . . It would appear, therefore, that there is not a great difference in toxicity of fluorine in different combinations when the amount required to produce the first discernible effect upon the tooth is the basis for comparison . . . It was found that a daily dose of 0.2 mg. of fluorine from any of the sources tested resulted in abnormal incisors of the 1-type . . . Barium fluosilicate in spite of its relative insolubility has been found in every case to equal sodium fluoride in toxicity when fed at the same level of fluorine . . . It has not been possible to demonstrate a contributing toxic effect of either barium or silicon."

A comparative study of the rate of retention and paths of excretion of fluorine when fed to rats as sodium fluoride, sodium fluosilicate, and calcium fluoride was made by Kick et al. (*13*). The data acquired are as follows:

	Fluorine source			
	$\mathrm{Na_2SiF_6}$	$\mathrm{Na_2SiF_6}$	NaF	$\mathrm{CaF_2}$
Number of rats	3	3	3	3
Approximate initial weight (gm.)	125	125	125	125
Days on experiment	23	22	18	11
Fluorine ingested—total mg	269. 9	269. 9	211. 2	229. 2
Fluorine ingested—daily mg	11. 7	12. 3	11. 7	20. 8
Fluorine in feces (mg.)	94. 3	94. 4	116. 5	225
Fluorine in feces (percent)	34. 94	34. 98	55. 16	98. 17
Fluorine in urine (mg.)	93. 6	90. 2	25. 8	4. 2
Fluorine in urine (percent)	34. 68	33. 42	12. 22	1. 83
Fluorine absorbed (mg.)	175 6	175. 5	94. 7	4. 1
Fluorine absorbed (percent)	65. 06	65. 02	44. 84	1. 78
Fluorine balance (mg.)	+82. 0	+85. 3	+68. 9	−00. 1
Fluorine balance (percent)	30. 38	31. 60	32. 62	. 04

In this instance there was no difference between sodium fluosilicate and sodium fluoride as regards the ultimate percent of fluorine retained in the rat's body, i.e., the percent fluorine balance in the above data. There were some differences, however, in the paths of excretion, i.e., in the urine or feces.

The metabolism of cryolite ($\mathrm{Na_3AlF_6}$), another complex fluoride, has been studied quite extensively (*14, 15, 16*) and the results may be of interest for comparison with the metabolism of $\mathrm{Na_2SiF_6}$. A study of the toxicity of fluorine in calcium fluoride and in cryolite (*14, 15*) thus brought out evidence that "the fluorine in cryolite is no more toxic to growing rats nor is it retained in the body to a greater extent than the fluorine in calcium fluoride, when both are administered in aqueous solution at the rate of 0.58 mg. per kilogram of body weight daily. . . . The appearance of striations in the incisor teeth is equally rapid with both fluorine compounds" (*14*). On the average approximately 59 percent of the ingested fluorine from both calcium fluoride and cryolite was retained by growing rats (*14*). In making this comparison of cryolite and calcium fluoride it was assumed by these authors that the fluorine in potable waters, insofar as it is in a nonionized condition, is in the form of calcium fluoride. In other experiments with cryolite, somewhat older rats retained the fluorine from synthetic cryolite, fine natural cryolite, and coarse natural cryolite in the following percentages, respectively: 36.14 percent, 32.03 percent, and 30.70 percent. Kick et al. (*13*), as already noted, also found about 30 percent of the fluorine in sodium fluosilicate, and sodium fluoride was retained by half-grown rats. The percent retention figures we obtained in this study agree with these later percentage figures (*13, 14*).

For a comprehensive review of sodium fluosilicate, its industrial uses and properties, a recent article by Hampel (*17*) should be consulted.

PLAN OF EXPERIMENT

One of the most pronounced biological effects of fluorine is its deposition in body tissues, particularly in bones and teeth. The body-retention of fluorine as offered to growing rats in the form of sodium fluoride and sodium fluosilicate is a reliable comparative criteria, therefore, of the physiological properties of these two chemically different fluorides. Specifically, in these experiments sodium fluoride and sodium fluosilicate were added to rats' drinking water in fluorine-equivalent quantities, and the concentration of fluorine in the rats' body tissues was determined.

Four series of experiments were completed as shown in tables 1, 2, 3, and 4 and in figures 1 and 2. In experiment 1 (table 1), groups of rats were fed ad libitum and drank water ad libitum) containing 5, 10, 15, 25, and 50 p.p.m. of fluorine added as sodium fluoride or sodium fluosilicate. After 90 to 100 days on the fluoride regimen, the rats were killed and the incisors, molar teeth, mandibles, and femurs were analyzed for ash, calcium, phosphorus, and fluorine. In this experiment and in the succeeding experiments, the rats' diet consisted of cornstarch 66.5 percent, whole milk powder 27.0 percent, yeast 5.0 percent, cod liver oil 1.0 percent, NaCl 0.5 percent, 10 p.p.m. of copper, and 100 p.p.m. of iron.

In experiment 2, 11 litter mate pairs of weanling rats were given drinking water containing 5.0 p.p.m. fluorine as sodium fluoride or sodium fluosilicate. The daily intake of drinking water was measured, so that each pair of rats ingested equal quantities of the respective fluorides. The rats were on experiment an average of 81 days. At the end of the experiment the rats were killed and the ash and fluorine content of the molar teeth, incisor teeth, and femurs were determined.

In experiment 3, the rats ingested 15 p.p.m. fluorine in drinking water. Over a period of 98-100 days, paired rats were given equal quantities of fluorine as sodium fluoride or sodium fluosilicate. In this experiment, food and water consumption was ad libitum but the water ingestion was measured. At the end of the experiment, the rats were killed and an analysis of ash and fluorine was made on the molar and incisor teeth and on the mandibles. The remaining body carcass was also analyzed for fluorine. In experiment 4, a comparison was made between sodium fluoride, sodium fluosilicate, and sodium fluoride plus sodium silicate using seven trios of rats. The fluorine in the drinking water was 25 p.p.m. The sodium fluosilicate solution contained 6.2 p.p.m. of silicon. Sodium silicate was added to sodium fluoride to furnish 15 p.p.m. of silicon in order to test the effect of silicon per se on the fluorine retention.

The molar teeth, incisor teeth, and the body carcass were analyzed for ash and fluorine.

In experiments 3 and 4, the measurements of total fluorine intake and subsequent analysis for total fluorine in the rat's body furnished data for a calculation of the total fluorine retained by the rats, i.e., the fluorine balance. Preparation of the rats' tissues for analysis was as follows: the digestive tract was removed and emptied of food residues, and the entire carcass autoclaved for 10 minutes at 15 pounds pressure. Incisor and molar teeth, mandibles, and femurs were removed and the remainder of the carcass was mascerated to a homogeneous pulp in a Waring Blendor.

Fluorine was determined in the teeth and bones by first extracting them with alcohol and ether drying, and ashing at 550° C. An aliquot sample of the homogenized carcass was dried and ashed at 550° C. using a CaO suspension as a fixative, and fluorine was determined on the ash. The fluorine in the ash was determined by the usual steam distillation from perchloric acid, evaporation of the distillate, and titration with standard thorium nitrate (*18*).

RESULTS OF EXPERIMENTS

In all these experiments on the growing rats, no different effects of sodium fluoride and sodium fluosilicate became apparent. Groups of rats ingesting fluorine in their drinking water ad libitum at levels of 5, 10, 15, 25, and 50 p.p.m. fluorine, as sodium fluoride and sodium fluosilicate (table 1), did not have different percentages of fluorine in the

TABLE 1.—*Comparison of fluorine ingested as* NaF *vs.* Na_2SiF_6 *by rats from drinking water and retention of fluorine and effect on ash, calcium, and phosphorus in bones and teeth*

	NaF	Na_2SiF_6	NaF	Na_2SiF_6	NaF	Na_2SiF_6	NaF	Na_2SiF_6	NaF	Na_2SiF_6	Averages	
											NaF	Na_2SiF_6
Fluorine in drinking water (ppm)	5	5	10	10	15	15	25	25	50	50		
Number of rats	20	20	17	19	19	20	17	23	23	27	(96)	(109)
Days on experiment	90	90	92	92	97	97	99	99	103	103	96	98
Initial weight (gm.)	30	30	30	30	31	30	32	30	30	30	30	30
Final weight (gm.)	122	115	131	134	139	148	145	162	148	148	137	143
Total gain (gm.)	92	85	101	104	108	118	113	132	118	118	107	113
Average daily gain (gm.)	1.0	0.9	1.1	1.1	1.1	1.2	1.1	1.3	1.1	1.1	1.1	1.2
	Results of tissue analyses in percentages											
Incisor teeth-ash	73.33	81.77	81.40	80.60	81.95	80.13	80.68	80.72	79.73	81.62	79.42	80.97
Fluorine in ash	.020	.017	.041	.030	.050	.065	.094	.086	.147	.129	.070	.06
Calcium in ash	33.70	34.89	34.18	34.66	34.65	34.45	33.47	32.84	34.80	34.44	35.62	34.26
Phosphorus in ash	19.53	19.13	19.12	20.25	18.58	19.82	19.62	20.08	18.72	20.52	19.11	19.96
Calcium to phosphorus in ash	1.73	1.82	1.79	1.71	1.86	1.74	1.71	1.64	1.86	1.68	1.70	1.72
Molar teeth-ash	75.37	77.96	76.63	76.92	76.05	75.72	77.14	76.95	76.82	77.36	76.40	76.96
Fluorine in ash	.039	.037	.061	.059	.092	.086	.110	.123	.170	.186	.095	.09
Calcium in ash	36.17	35.77	35.41	36.84	35.33	35.05	36.47	36.07	35.34	35.50	35.74	35.85
Phosphorus in ash	18.37	17.76	18.15	18.63	18.12	18.90	18.27	17.41	17.57	16.92	18.10	17.92
Calcium to phosphorus in ash	1.97	2.01	1.95	2.00	1.95	1.85	2.00	2.07	2.01	2.10	1.98	2.00
Mandibles-ash	67.70	70.71	69.88	68.52	70.17	69.29	69.99	69.24	69.49	70.33	69.45	69.62
Fluorine in ash	.062	.057	.104	.096	.163	.161	.216	.220	.347	.329	.178	.17
Calcium in ash	36.23	37.17	36.14	36.06	36.75	37.25	35.56	37.01	37.17	36.21	36.37	36.74
Phosphorus in ash	18.00	18.76	17.71	18.22	17.35	16.96	18.00	17.46	18.31	18.58	17.87	18.00
Calcium to phosphorus in ash	2.01	1.99	2.04	1.98	2.12	2.20	1.98	2.12	2.03	1.95	2.04	2.04
Femur-ash	58.85	62.77	62.23	59.28	64.20	65.22	63.65	66.19	62.32	67.35	62.25	64.16
Fluorine in ash	.077	.063	.126	.116	.186	.182	.270	.281	.424	.393	.217	.207
Calcium in ash	36.72	34.85	36.05	36.36	37.65	37.06	36.52	36.27	37.77	36.62	36.94	36.23
Phosphorus in ash	18.78	18.24	18.26	18.20	18.94	18.44	18.46	17.78	18.48	17.97	18.58	18.13
Calcium to phosphorus in ash	1.96	1.91	1.97	2.00	1.99	2.01	1.98	2.04	2.04	2.04	1.99	2.00

ash of the bones and teeth which could be related to the kind of fluoride fed. Similarly, no differences occurred in the percent of ash calcium and phosphorus and the calcium to phosphorus ratio in the molar and incisor teeth, mandibles, and incisors. Average daily gain in body weight was similar in all groups of rats.

The data in table 2 were obtained by equalizing the drinking water ingested by pairs of rats over a period of 81 days, so that the total quantity of fluorine was the same for both rats of a pair. The average results for 11 pairs of rats receiving 5 p.p.m. fluorine in their drinking water are shown in table 2. Here again it is apparent that the percent of fluorine in the ash of the molar teeth, incisor teeth, and femurs was not related to the source of fluorine, i.e., sodium fluoride or sodium fluosilicate.

The fluid intake was measured, and the final total quantity of drinking fluid (and thus the total fluorine ingested) was equalized in 10 pairs of rats. The average results of this study appear in table 3. The amount of fluorine in the drinking water was 15 p.p.m.. No differences in the ash and the fluorine content of the ash of teeth and mandibles occurred. The fluorine retained in the rat's entire body amounted to 33.10 percent of the total ingested as sodium fluoride, and 36.06 percent of the total ingested as sodium fluosilicate. The difference between these two percentage figures is not significant. In these 10 paired results for sodium fluoride vs. sodium fluosilicate the mean difference in percent retention was 2.96 with a standard error of ±6.56.

In table 4 are the results of adding 25 p.p.m. fluorine to the drinking water as sodium fluoride, sodium fluosilicate, and sodium fluoride plus sodium silicate. No differences appear in the results for ash and fluorine in the molar and incisor teeth. While the percent of ingested fluorine which was retained by the rats receiving sodium fluoride plus sodium silicate amounted to 33.09 percent as compared with 38.36 percent retention of fluorine ingested as sodium fluoride alone, the difference between these two values is not statistically significant. The mean difference for these seven paired comparisons was 5.27; the standard error was ±5.89.

Similarly, there is no significance in the difference, 38.36 percent vs. 41.49 percent, i.e., the percent fluorine retained from sodium fluoride vs. sodium fluosilicate

Figure 2 shows the relations between the fluorine in the ash of incisors, molars, mandibles, and femurs at different levels of fluorine in drinking water. As would be expected, these quantities are proportional to the fluorine ingested and the similarity between the two

TABLE 2.—*Comparison of* NaF *vs.* Na_2SiF_6 *as a source of 5 p.p.m. fluorine in rats' drinking water (average data for 11 pairs of rats in experiment 2)*

Experiment data	Fluorine exposure	
	NaF	Na_2SiF_6
Total fluorine intake (mg.)	5. 4	5. 4
Initial body weight (gm.)	39. 4	36. 8
Total gain in weight (gm.)	153. 2	142. 5
Days on experiment	81. 0	81. 0
Average daily gain (gm.)	1. 89	1. 76
Molar teeth analysis		
Total weight (mg.)	113. 6	135. 0
Percent ash	76. 91	76. 85
Percent fluorine in ash	. 029	. 032
Total fluorine (mg.)	. 025	. 033
Incisor teeth analysis		
Total weight (mg.)	249. 4	235. 8
Percent ash	74. 57	74. 62
Percent fluorine in ash	. 017	. 018
Total fluorine (mg.)	. 032	. 031
Femur analysis		
Total weight (mg.)	699. 1	616. 4
Percent ash	61. 38	64. 38
Percent fluorine in ash	. 070	. 065
Total fluorine (mg.)	. 301	. 259

TABLE 3.—*Comparison of* NaF *and* Na_2SiF_6 *as a source of 15 p.p.m. of fluorine in rats' drinking water (average data for 10 pairs of rats)*

Experiment data	Fluorine exposure	
	NaF	Na_2SiF_6
Total fluorine intake (mg.)	19. 8	20. 2
Initial body weight (gm.)	36. 9	38. 8
Total gain in weight (gm.)	165. 7	168. 2
Average daily gain (gm.)	1. 69	1. 68
Molar teeth analysis		
Total weight (mg.)	131. 7	130. 1
Percent ash	77. 18	76. 72
Percent fluorine in ash	. 087	. 093
Total fluorine (mg.)	. 088	. 093
Incisor teeth analysis		
Total weight (mg.)	245. 5	257. 5
Percent ash	77. 88	76. 85
Percent fluorine in ash	. 062	. 066
Total fluorine (mg.)	. 118	. 130
Mandibles analysis		
Total weight (mg.)	307. 7	311. 1
Percent ash	69. 98	69. 93
Percent fluorine in ash	. 145	. 153
Total fluorine (mg.)	. 313	. 332
Fluorine in carcass* (mg.)	6. 035	6. 729
Total fluorine retained (mg.)	6. 553	7. 284
Total percent fluorine retained	33. 10	36. 06

*Minus mandibles, molars, and incisors

fluorides ingested is shown in the percentage figures. There is a slightly greater percent of fluorine in the ash of the femurs, than in the ash of mandibles, but there is also less ash in the femurs than in the mandibles. The percent fluorine in the fat-free mandibles and in the fat-free femurs is essentially the same.

The appearance of the characteristic striations in these rats' incisor teeth did not show variations which could be related to the kind of fluoride ingested. The striations varied with the level of fluorine in the drinking water and were most apparent in the lower incisors. At a level of 10 p.p.m. fluorine, incisor striations were easily apparent under low power magnification. At 5 p.p.m. fluorine in the drinking water, striations could not be seen, but there was some tendency toward slight depigmentation in several rats. Considerable spotty depigmentation occurred at 25 p.p.m. fluorine and over.

The average rate of growth of the various groups of rats is indicated by the growth curves shown in figure 1. The essential similarity in rate of growth for all groups of rats suggests no differences in the rats' reaction to sodium fluoride vs. sodium fluosilicate. The presence of sodium silicate in the drinking water (experiment 4) also had no effect on growth.

These results are in general agreement with the previous evidence of similar physiological effects of these two fluorine compounds. While the similarity in the quantities of fluorine retained in the teeth and skeletal tissues is the most pertinent evidence obtained in these experiments, the other data relative to ash, calcium and phosphorus, and the calcium to phosphorus ratio of bones and teeth, the rate of growth, and the appearance of characteristic striations in the rats' incisors, also contribute evidence that similar physiological effects result from these two fluorides.

As additional data are obtained, particularly with reference to dental caries effects, conclusions may be warranted regarding the possibility of substituting sodium fluosilicate for sodium fluoride, as a water fluoridizing agent. The data accumulated thus far, however, do not justify any commitments in this regard.

Table 4.—*Comparison of* NaF, Na_2SiF_6, *and* $NaF+Na_2SiO_3$ *as a source of 25 p.p.m. fluorine in rats' drinking water (average data for 7 trios of rats)*

Experiment data	Fluorine exposure		
	NaF	Na_2SiF_6	$NaF+Na_2SiO_3$
Total fluorine intake (mg.)	33. 0	31. 5	33. 3
Initial body weight (mg.)	38. 4	36. 1	44. 4
Total gain in weight (gm.)	137. 9	159. 6	161. 0
Days on experiment	107. 0	105. 0	106. 0
Average daily gain (gm.)	1. 29	1. 52	1. 51
Molar teeth analysis			
Total weight (mg.)	126. 5	134. 2	137. 5
Percent ash	77. 16	77. 23	77. 72
Percent fluorine in ash	. 178	. 176	. 187
Total fluorine (mg.)	. 173	. 183	. 220
Incisor teeth analysis			
Total weight (mg.)	263. 2	261. 1	276. 8
Percent ash	78. 28	77. 91	77. 36
Percent fluorine in ash	. 101	. 101	. 103
Total fluorine (mg.)	. 208	. 204	. 221
Fluorine in carcass* (mg.)	12. 29	12. 69	10. 58
Total fluorine retained (mg.)	12. 66	13. 07	11. 02
Total percent fluorine retained	38. 36	41. 49	33. 09

*Minus molar and incisor teeth.

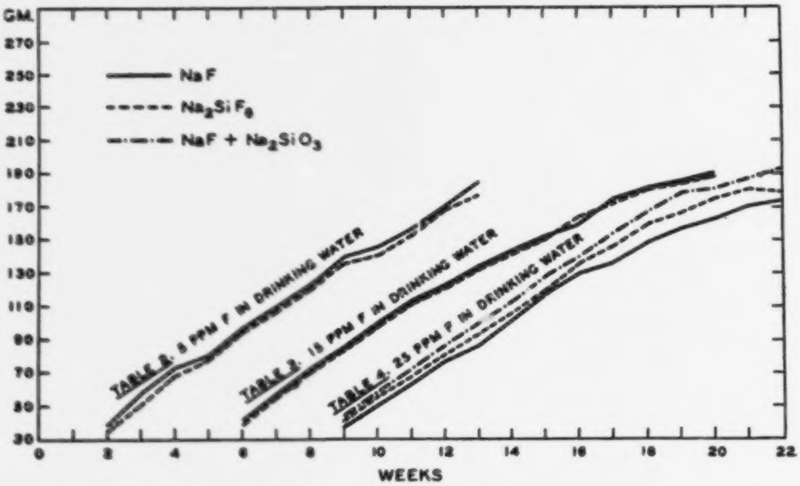

Figure 1.—*Average rate of growth of rats receiving sodium fluoride, sodium fluosilicate, and sodium fluoride plus sodium silicate in drinking water.*

SUMMARY

1. A comparison was made of the effect of fluorine ingested by rats from drinking water in the form of sodium fluoride vs. sodium fluosilicate; the quantity of fluorine equaled 5, 10, 15, 25, and 50 p.p.m.

2. No differences were observed in the quantity of fluorine deposited in the incisor and molar teeth, mandibles, and femurs, nor in the percent of the ingested fluorine which was retained in the rat's body. There was no difference in the ash, calcium, and phosphorus content of the incisor teeth, molar teeth, mandibles, and femurs which could be related to the kind of fluoride ingested.

3. There were no differences in the appearance of the characteristic striations on the rats' incisor teeth which could be attributed to the sodium fluoride vs. sodium fluosilicate.

4. The presence of 15 p.p.m. of silicon as sodium silicate along with 25 p.p.m. of fluorine as sodium fluoride did not affect the amount of fluorine deposited in the rat's body.

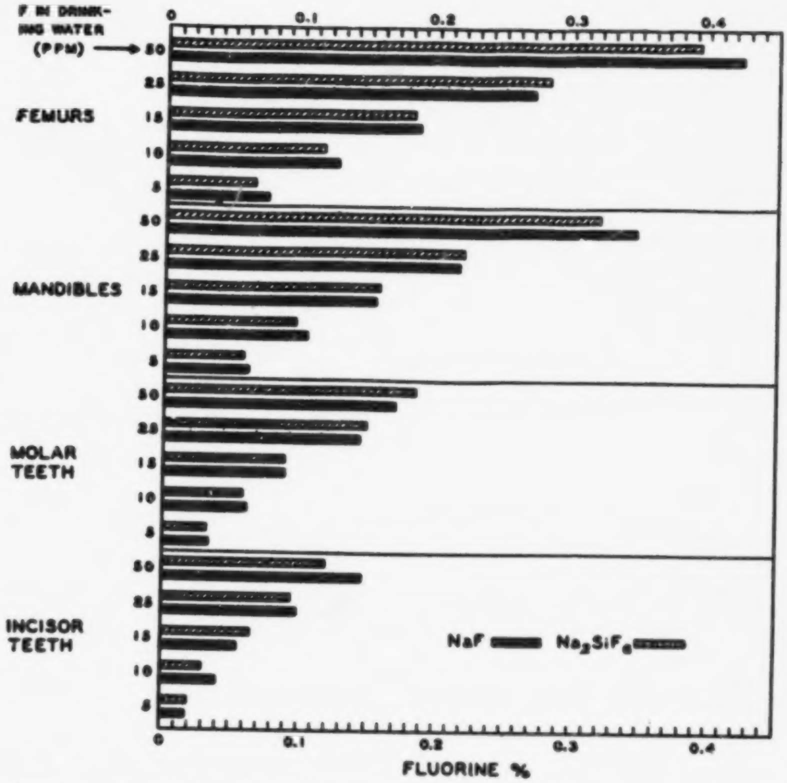

FIGURE 2.—*Fluorine in ash of bones and teeth of rats receiving fluorine as sodium fluoride or sodium fluosilicate in drinking water.*

5. The rate of growth was normal in all groups of rats.

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DEPOSITION OF FLUORINE IN THE BONES AND TEETH OF THE GROWING RAT

I. Zipkin and F. J. McClure

Only a limited number of reports have appeared on the effect of age on fluorine retention in bones and teeth, either when the fluorine exposure is continuous throughout life, or when it is instituted at various ages during the life span.

Lawrenz, Mitchell and Ruth (1940) found that "growing rats adapt themselves to the continuous ingestion of low levels of fluorine by excreting greater and greater proportions of the ingested fluorine in feces and urine," indicating that less fluorine was retained by aging skeletal and dental tissues. The fluorine excretion appeared also to approach a maximum or plateau value, which suggests a stabilized concentration of fluorine in the calcified tissues and a reduction in their capacity for fluorine retention.

A diminishing retention of skeletal fluorine with increasing age is also apparent from the data of Ellis and Maynard (1936). Over a 168-day period during which 8 p.p.m. fluorine were added to the diet, approximately 70 percent of the fluorine in rats' "leg bones" accumulated during the first third (56 days) of the fluorine exposure period. In another comparison in which 12 p.p.m. fluorine were added to the rats' diet, 55 percent of the total fluorine accumulated during the first third (56 days) of the exposure period.

Glock et al. (1941) fed approximately 200 p.p.m. fluorine in the diet to 6-week-old rats for 90 weeks, sacrificed representative rats at irregular intervals and analyzed the "long bones" for fluorine. The data fitted a logarithmic curve of diminishing increment, suggesting that a constant level of fluorine retention is attained with high as well as low levels of intake of fluorine.

Jackson, Tisdall, Drake and Wightman (1950) administered a diet containing approximately 5 p.p.m. fluorine to rats, sacrificed a representative number at 6-, 12-, and 18-month intervals and found that the total fluorine per rat carcass was essentially the same at each sacrifice period. Apparently fluorine was built up to a constant level and maintained so long as the dietary level was unchanged.

Data on the retention of fluorine in the calcified tissues in rats exposed to a given quantity of fluorine at various ages with no prior exposure to fluorine, appear to be limited to the work of (*a*) Jackson et al. (1950), who found that 6-month-old rats retained considerably less fluorine in the carcass than young rats started at 6 to 8 weeks of age, and (*b*) Savchuck and Armstrong (1951), who found that rats put on a fluorine regimen at 92 days of age retained less fluorine in their humeri than rats started at 27 days of age.

It was the purpose of this investigation to make a more comprehensive study of the effect of age on fluorine retention by means of periodic fluorine analyses of femurs, mandibles and teeth of rats undergoing continuous fluorine ingestion from 30 days to 450 days of age, and also by similar analyses to determine the response of the bones and teeth to fluorine introduced at various ages in the life of rats without prior exposure to fluorine.

PLAN OF THE EXPERIMENT

The investigation was composed of two studies, each of which was divided into 5 periods. At the start, Sprague-Dawley rats were housed 9 to a cage and received diet 550, of the following composition: corn starch 66.5; whole milk powder 27.0; yeast 5.0; cod liver oil 1.0; and salt mixture 0.5 percent. The salt mixture contained 500 gm. of NaCl, 53.2 gm. of ferric citrate trihydrate and 3.9 gm. of copper sulfate pentahydrate.

In study I, the control rats received distilled water ad libitum, and the experimental rats received 10 p.p.m. F as NaF ad libitum in the drinking water, starting at 30 days of age.

Control and experimental rats were sacrificed after 60, 120, 180, 300 and 420 days on experiment. The femurs and mandibles of 6 control rats and 6 experimental rats, selected at random, were removed for analysis. The molar teeth of approximately 20 control rats were pooled, whereas the molar teeth of each experimental rat were sufficient for analysis. Fluorine analyses were made according to standard procedures (Willard and Winter, 1933; McClure, 1939) on the alcohol- and ether-extracted bones and teeth, dried overnight at 105°C.

In study II, control rats were fed the stock diet individually throughout the experiment. Starting at 30, 90, 150, 210 and 330 days of age, their drinking water contained 10 p.p.m. of fluorine as NaF. The fluid intake was measured and when 2,000 ml. or 20 mg. fluorine had been ingested, six rats most closely approximating the mean weight of the group were sacrificed.

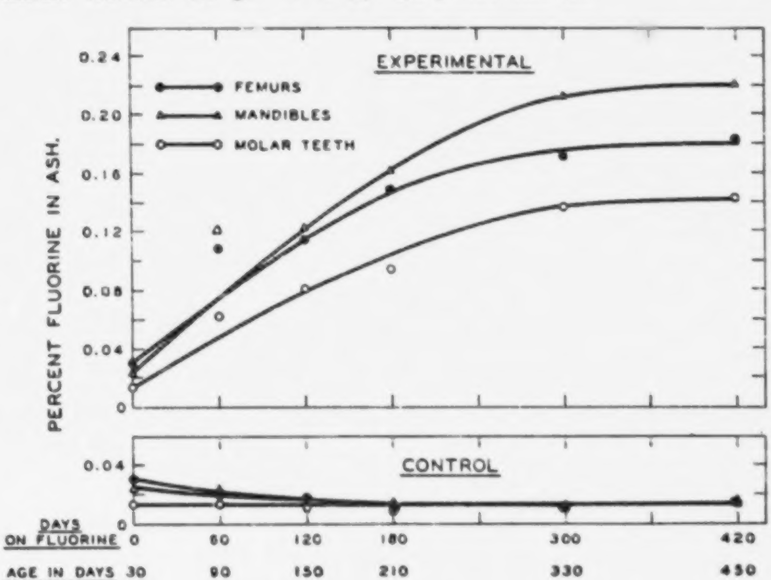

Figure 1.—*Percent fluorine in the ash of the bones and teeth of growing rats receiving 10 p.p.m. fluorine as NaF for 420 days starting at 30 days of age.*

The molar teeth, femurs and mandibles were removed and analyzed for fluorine as previously noted. Aliquot samples of the femurs and mandibles of the rats of studies I and II were ashed and analyzed for calcium (Association of Official Agricultural Chemists, 1950a) and phosphorus (A.O.A.C., 1950b).

RESULTS

Study I

To establish the initial fluorine content, a pooled sample of molar teeth, femurs and mandibles of 30-day-old rats was analyzed for ash and fluorine. The relationship of the percent fluorine in the ash of the molar teeth, femurs, and mandibles to duration of fluorine exposure is presented graphically in figure 1 and similar data for total fluorine are presented graphically in figure 2.

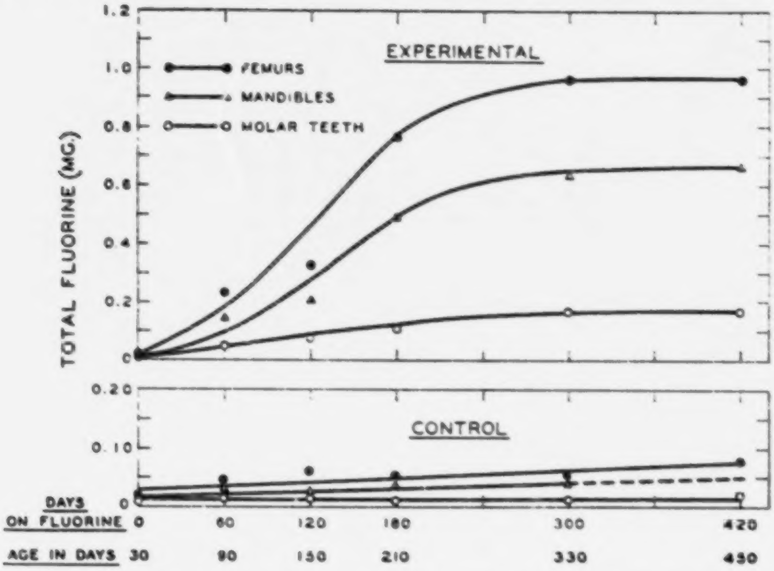

Figure 2.—*Fluorine content of the bones and teeth of growing rats receiving 10 p.p.m. fluorine as NaF for 420 days starting at 30 days of age.*

In general, there was only a slight change in the percent fluorine in the ash of control rats' bones and teeth during study I. The small increases in total fluorine in these tissues were due mainly to normal weight gains. As is shown in figure 2, those control rats which received only 1 p.p.m. fluorine from their basal diet increased their total fluorine twofold, fourfold, and threefold in the molar teeth, femurs, and mandibles, respectively, by the end of the 450 days.

As is shown in figures 1 and 2, both the percent fluorine and the total fluorine of the molar teeth, mandibles, and femurs of the experimental rats underwent marked increases up to 330 days of age; i.e., 300 days on a fluoride regimen of 10 p.p.m. fluorine in their drinking water. Further deposition of fluorine, however, was not apparent at the end of 450 days. Apparently a stabilized or saturation level of fluorine in the rats' calcified tissues was reached at about 330 days of age; i.e., near the end of the period of rapid growth.

By the end of 450 days of age, at which time the rat has already reached maturity, the total fluorine in the test rats had multiplied 20 times, 50 times, and 40 times in the molar teeth, femurs, and mandibles, respectively, as compared with their initial fluorine content. These would appear to represent maximum increases in fluorine under these conditions of intake at a level of 10 p.p.m. in the drinking water.

During the experimental period of 420 days there was no appreciable change in the calcium and phosphorus content of the femurs and mandibles of the rats receiving 10 p.p.m. fluorine. The calcium content of the femur and mandible ash averaged 39.29±1.16 percent and 39.09±1.11 percent, respectively, and the phosphorus content averaged 18.82±0.79 percent and 18.88±1.13 percent, respectively.

Study II

The purpose of study II was to observe the deposition of fluorine in the molar teeth, femurs and mandibles of rats receiving equal amounts of fluorine as sodium fluoride at different ages, with no previous fluorine exposure. These data are presented in figures 3 and 4.

A pronounced decrease was apparent in the percent and total fluorine deposited in bones and teeth with increasing age; i.e., old rats without prior exposure to fluorine stored considerably less fluorine in their bones and teeth than younger animals. The ratios of the percent and total fluorine found in the bones and teeth of the rats of group V (oldest rats) to those of the rats of group I (youngest rats) were, respectively, as follows: for molar teeth, 0.22 and 0.20; for femurs, 0.37 and 0.48; and for mandibles, 0.32 and 0.34.

It is of interest to note also that the femurs and mandibles contained a similar percent of calcium and phosphorus, and this remained unchanged despite a variable fluorine content. Thus the femurs and mandibles averaged 38.85±1.74 percent calcium and 39.03±1.42 percent calcium, respectively, and the phosphorus content averaged 18.43±0.81 percent and 18.56±0.70 percent, respectively.

DISCUSSION

The data obtained in both study I and study II agree with previous evidence and emphasize the fact that the animal body, as typified by the rat, does not store fluorine with fixed regularity and constancy throughout life. Retention of fluorine by the bones and teeth in these rats appeared to be primarily related to growth; i.e., the fluorine deposition curves shown in figures 1 and 2 follow the general pattern of a normal growth curve. During the period of rapid growth, both the percent and total fluorine of the molar teeth, femurs and mandibles increase at a relatively rapid rate. In the mature rat, however, fluorine storage does not increase with increase in age, as shown by essentially similar concentrations of fluorine in the bones and teeth of rats at 330 and 450 days of age. It appears that the data of figures 1 and 2 describe a relationship of diminishing increment between fluorine deposition and time. This is in accord with the data of Lawrenz et al. (1940), previously referred to.

Interpolation from the curves of figure 2 shows that 50 percent of the fluorine retained by the molar teeth over a 420-day experimental period was accumulated during the first third (120 days) of the fluorine exposure period. During this same interval, the mandibles and femurs accumulated 57 percent and 66 percent of the total fluorine retained. The latter value for femurs is in good

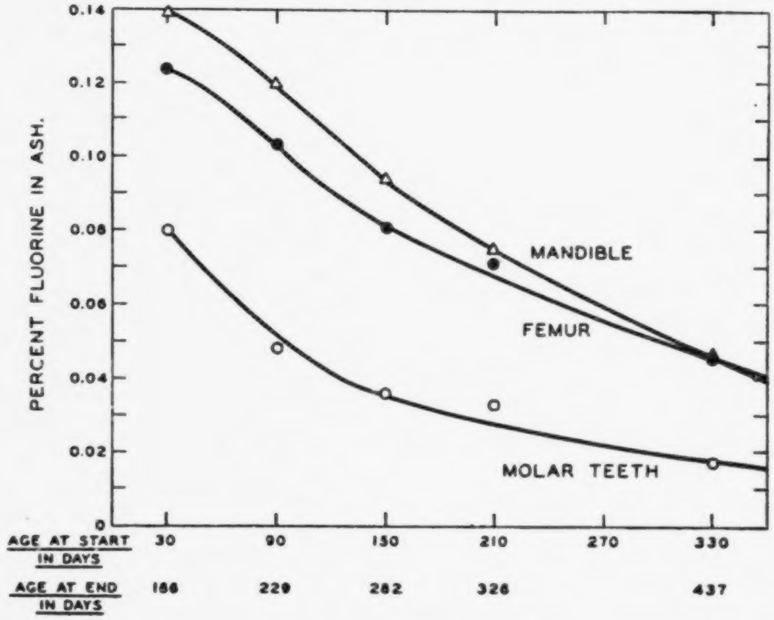

FIGURE 3.—*Percent fluorine in the ash of the bones and teeth of growing rats receiving 20 mg. of fluorine as NaF at different ages, with no prior exposure to fluoride.*

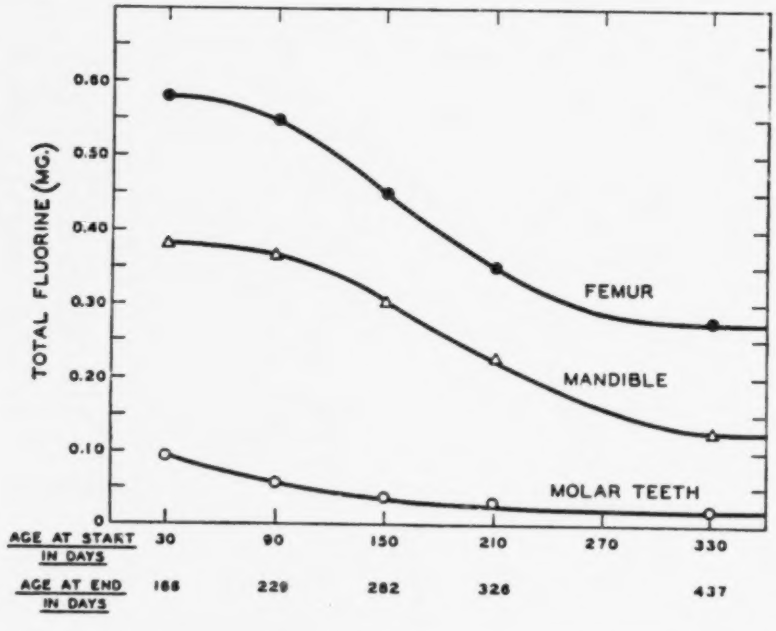

FIGURE 4.—*Fluorine content of the bones and teeth of growing rats receiving 20 mg. of fluorine as NaF at different ages, with no prior exposure to fluoride.*

accord with the data of Ellis and Maynard (1936), who found that approximately 70 percent of the fluorine in the rats' "leg bones" accumulated during the first third of the fluorine exposure period when 8 p.p.m. fluorine were added to the diet.

Although the rats in study II had no prior fluorine exposure, as contrasted with the continuous fluorine exposure of the animals of study I, these rats (study II) showed a similar loss of ability to retain fluorine with advancing age.

By reference to figures 2 and 4 it is possible to estimate the effect of prior exposure to fluorine on its subsequent deposition. As shown in figure 2, only a very slight amount of fluorine is deposited in the bones and teeth of rats during the interval between 330 and 437 days of age when the animals receive 10 p.p.m. F continually throughout life. During this same interval of time, rats with no prior exposure to fluorine accumulated appreciable amounts in the femurs and mandibles, as is shown in figure 4, when compared to the fluorine deposition in the control rats, as shown in figure 2.

Thus the 330-day-old rats of study II (no prior exposure to fluorine) stored 0.22 mg. and 0.09 mg. in the femurs and mandibles, respectively; whereas similar rats of study I (continuous exposure to fluorine) stored only 0.02 and 0.01 mg. of fluorine, respectively.

The molar teeth of rats 330 days of age with no prior exposure to fluorine and receiving 10 p.p.m. fluorine contained the same amount of fluorine at 437 days of age as those of control rats of the same age.

These observations indicate, therefore, that the bones of the mature rats in study I were exhausting their fluorine deposition potential, whereas rats of similar age (study II) with no prior exposure to fluorine retained their ability to store fluorine, albeit at a much reduced capacity compared to that of rapidly growing rats. It is interesting to note that a similar situation has been demonstrated with regard to skeletal deposition of radioactive calcium in young and adult rats by Copp et al. (1951) and Hansard et al. (1951), who showed that skeletal uptake of calcium 45 is greater in growing rats than in older ones.

SUMMARY

Rats 30 days of age received 10 p.p.m. F as sodium fluoride for a period of 420 days. At various intervals during this period representative rats were sacrificed and the molar teeth, femurs and mandibles were analyzed for fluorine. It appears that the growing rat shows a diminishing ability to store fluorine in the bones and teeth with advancing age and after the period of rapid growth accumulates little or no additional fluorine.

Rats put on a fluoride regimen at various ages of from 30 to 330 days

without prior exposure to fluorine, and receiving equal quantities of fluorine, also showed a pronounced decreasing capacity to incorporate fluorine in the bones and teeth with advancing age.

The results agree with previous evidence that the animal body accumulates fluorine at a comparatively rapid rate during growth, but reaches a relatively constant level of skeletal fluorine at maturity. This is maintained throughout the remaining years of life so long as the fluorine intake remains stabilized.

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FLUORIDE DEPOSITION IN DIFFERENT SEGMENTS OF THE TIBIA OF THE YOUNG GROWING RAT

I. Zipkin and R. O. Scow

ABSTRACT

By inserting a stainless steel pin into the tibiae of young rats, it was possible to determine the gain in length at each end of the diaphysis and to identify the bone that was deposited at the ends during the experimental period (20th–51st day of life). At the termination of the experiment, the tibiae were divided into four segments, three of which contained portions of the diaphysis. The oven-dry, fat-free weight, ash and fluoride content of these segments were determined. Intraperitoneal injections of 3.15 mg. of fluoride (NaF) over a 30-day period, starting at 20 days of age, had no noticeable effect on the longitudinal growth rate and mass of the various tibial segments. The ash content of the various diaphyseal segments was similarly unaffected. The percentage of fluoride (ash basis) of the proximal diaphyseal segment (all new bone) was similar to that of the middle diaphyseal segment, but 35 percent lower than that of the proximal epiphysis. There appears to be no consistent relationship between the degree of calcification (percent ash) and the deposition of fluoride in various tibial segments at the end of 30 days in the young growing rat.

The skeleton has been recognized as the principal site of deposition of fluoride in the rat (*1*). It has been demonstrated that the calcified tissues of the rat accumulate fluoride at the greatest rate during the period of most active growth (*2*). As is well known, increase in length of a long bone occurs by appositional growth at the proximal and distal epiphyseal lines (endochondral ossification). Although several reports have appeared on the deposition of fluorine in different parts of the same bone (*3–5*), no correlation has been made with their rate of growth.

In the present study, the rate of longitudinal growth of a long bone was measured by inserting a metal marker into the tibia (*6–8*). Thus, by using this technique it was possible to identify the entirely new bone immediately distal to the proximal epiphyseal plate, and to compare the deposition of fluoride therein with that in adjacent segments of the diaphysis. In addition, the effect of a low level of fluoride on the rate of longitudinal growth of the tibia was determined.

EXPERIMENTAL

Twenty-four, 20-day old, male, Sprague-Dawley rats weighing 32–38 gm. were divided equally by weight into a control and a fluoride-treated group. Under ether anesthesia, a stainless steel pin approximately 0.5 mm. in length was inserted into the middle third of the anteromedial aspect of the left tibia. The position of the pin in the tibia is shown in figure 1. In the present study the tibia was chosen for study since, in the rat, it increases in length at a greater rate than the femur (*9*).

The treated rats received 21 intraperitoneal injections of 0.5 ml. of a sodium fluoride solution containing 300 p.p.m. F. Each rat was injected on weekdays only for a period of 31 days and hence received 3.15 mg. of fluoride which would approximate the daily intake of rats of similar age drinking water containing 10 p.p.m. F for a similar length of time. Distilled water and a stock

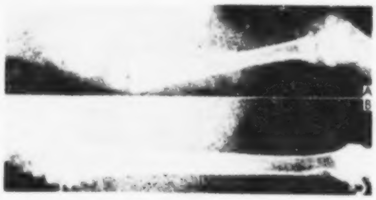

FIGURE 1.—*Roentgenograms showing the position of the stainless steel pin in the left tibia of the same rat at A, 20 days and B, 51 days of age. The pin was inserted at 20 days of age* (× 3).

diet containing 1 p.p.m. F (*2*) were available to all rats ad libitum. The animals were roentgenographed at the start and at 12, 20 and 31 days thereafter or at 20, 32, 40 and 51 days of age to assess the longitudinal rate of growth of the tibia as shown in figure 2. A similar group of twelve control rats were roentgenographed at 112 days of age.

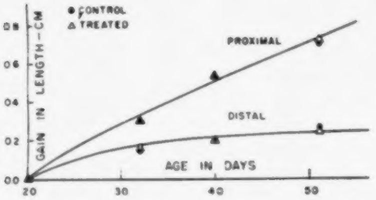

Figure 2.—*Increase in length at the proximal and distal ends of the tibal diaphysis of fluoride-treated and control male rats. The average length of the tibial diaphysis in 20-day-old male rats was 1.65 cm.*

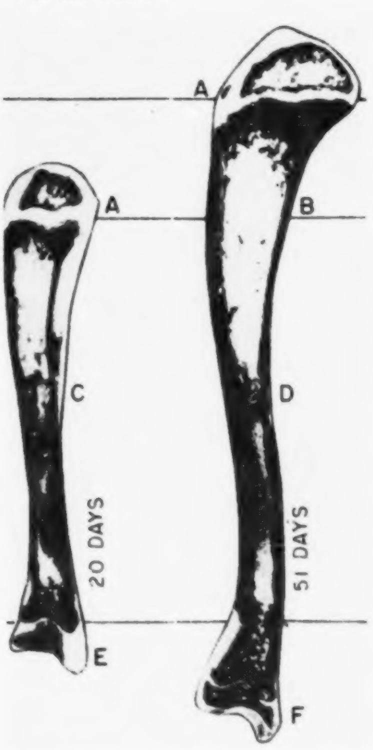

Figure 3.—*Photograph of a sagittal section through the tibia of a 20- and 51-day-old rat to illustrate the segments obtained for analysis. Stained with silver nitrate (10) (× 3). A—proximal epiphysis. B—proximal diaphyseal segment. C—diaphysis. D—middle diaphyseal segment. E—distal epihysis. F—distal diaphyseal segment plus distal epiphysis.*

For each rat, the gain in length of the tibial diaphysis at each end was determined by measuring the distance from the proximal end of the pin to the proximal and distal epiphyseal lines of the left tibia on the roentgenograms taken at the intervals stated.

After 31 days, the rats were killed, the tibiae were excised and freed of adhering tissue. The increment at each end was measured from the respective epiphyseal lines and marked off on both tibiae. The tibia was then divided with a razor blade at the two lines of demarcation on the diaphysis and through the proximal epiphyseal line as shown in figure 3.

Each tibia of the 51-day old rats was thus divided into four segments as follows: (*a*) the proximal epiphysis, (*b*) the proximal diaphyseal segment which consisted only of bone added to the proximal end of the original diaphysis, (*c*) the middle diaphysis, which represented the length of the diaphysis present at the beginning of the experiment, and (*d*) the distal segment. The distal segment consisted of both the distal epiphysis and the bone added to the distal end of the diaphysis (the distal diaphyseal segment). The distal segment was not sectioned through its epiphyseal disc since this procedure would produce segments of insufficient mass to warrant handling in the analytical procedures. In addition, our interest centered on the relative deposition of fluoride in those segments of the tibia showing the greatest and the least longitudinal increment; that is, the proximal and middle diaphyseal segments, respectively.

The oven-dry, fat-free weight and ash content of the epiphyses and diaphysis of the tibiae of 20-day-old male, Sprague-Dawley rats weighing 32–38 gm. were also determined.

To insure sufficient sample for analysis, it was necessary to pool a number of similar segments from several tibiae as shown in table 1. All samples were dried at 105° C, extracted in a Soxhlet with alcohol and ether for 8 and 4 hours, respectively, ashed at 550° C for 3 hours, and analyzed for fluoride according to standard procedures (*11, 12*).

RESULTS

The presence of the pin in the tibial shaft had no effect on the growth of the tibial diaphysis. In the 12 control rats during 31 days, the gain in length of the left tibial diaphysis with pin averaged 0.98±0.03 cm. and the right tibial diaphysis without pin averaged 0.95±0.08 cm. In 11 fluoride-treated rats the gain in length was 0.96±0.03 and 0.96±0.02 cm. respectively.

After 31 days on experiment, the increment in length of the diaphysis at the proximal end was approximately 2.5 times that at the distal end (fig. 2). At this time there was no appreciable further increase at the distal end, whereas the diaphysis continued to grow at the proximal end. The 112-day-old rats showed a gain in length of 1.02 cm. at the proximal end of the diaphysis. The gain in length for the 51-day-old rats was 0.71 cm. Fluoride had no effect on the rate of growth at either end of the tibial diaphysis.

As may be seen from table I, there were no significant differences in the oven-dry, fat-free weight of similar segments of the 51-day-old control and fluoride-treated rats. Although the diaphysis of the 20-day-old rats is the same length as the middle diaphyseal segment of the 51-day-old rats as shown in figure 3, there is a significant increase in the weight of the latter ($t=2.95$, $P < 0.02$) thus indicating extensive deposition of bone salt.

As may be seen from the table, the highest ash content in the 20-day-old rats was found in the diaphysis and the lowest in the proximal epiphysis. It

Table I.—*Analysis of tibial segments* *

Age, days	Proximal Epiphysis	Proximal Diaphyseal Segment	Middle Diaphyseal Segment	Distal Diaphyseal Segment and Distal Diaphysis
		Oven dry, fat free weight, mg†		
20	10.0 ± 5.7 (2)			
51 (co)	23.2 ± 0.6 (3)	33.0 ± 1.9 (6)	44.6 ± 3.8 (6)	24.1 ± 1.2 (3)
51 (tr)	23.2 ± 4.9 (3)	34.9 ± 2.0 (6)	45.4 ± 3.9 (6)	22.6 ± 3.8 (3)
		Ash content, % of oven-dry, fat-free weight‡		
20	23.1 ± 0.31 (2)			
51 (co)	36.4 ± 0.36 (3)	49.0 ± 0.60 (6)	64.0 ± 0.16 (6)	47.8 ± 0.42 (3)
51 (tr)	34.5 ± 0.34 (3)	47.3 ± 0.48 (6)	63.8 ± 0.23 (6)	47.4 ± 0.46 (3)
		Fluoride content, % of ash		
51 (co)	0.006 (1)	0.005 ± 0.0005 (2)	0.009 ± 0.0003 (2)	0.009 (1)
51 (tr)	0.132 ± 0.002 (3)	0.097 ± 0.002 (6)	0.098 ± 0.002 (6)	0.120 ± 0.003 (3)

* All data are expressed as mean ± S.E. Numbers in parentheses refer to number of pooled aliquots analyzed

† At 20 days: diaphysis 28.1 ± 4.1 (4) mg; distal epiphysis 6.8 ± 0.4 (2) mg.

‡ At 20 days: diaphysis 52.0 ± 0.32 (4)%; distal epiphysis 28.7 ± 0.12 (2)%.

should be noted that in the 51-day-old rats the ash content of the middle diaphyseal segment is higher than that of the proximal diaphyseal segment. This finding is to be expected since the latter segment represents entirely new bone formed during the experiment. There is a significant increase in the ash content of the middle diaphyseal segment of the 51-day-old rats over that in the diaphysis of the 21-day-old rats ($t=3.08$, $P < 0.02$), again indicating considerable increase in the mineral components. No differences in the percentage of ash were found between similar segments of the control and fluoride rats, except for the proximal epiphysis. Although the percentage of ash was higher in this segment in the control rats at the 2 percent probability level, the small number of cases precludes any conclusive evaluation of this difference.

The fluoride content of the various tibial segments of 51-day-old rats is presented in table 1. When expressed as percentage of ash, the fluoride concentrations of the proximal epiphysis and of the distal segment were similar and were 35 percent higher than those of the proximal and middle diaphyseal segments.

DISCUSSION

It is difficult to accurately assess the rate of growth of a long bone. In addition to increase in length there is an increase in width which has been measured by Leblond *et al.* using P^{32} (*13*). The cortex of the diaphysis is highly vascular (*14*) and undergoes considerable reorganization in a short period of time (*13*). Thus, addition of bone to the outside of the diaphysis (periosteal ossification) and a corresponding resorption on the inside results in widening of the diaphysis. The degree to which each process contributes to the growth of a long bone is at present difficult to evaluate.

In the present study the proximal diaphyseal segment consisted only of bone formed during the experimental period. This bone was primarily the result of very active endochondral ossification (*14*) which was still active at the end of the experiment as seen by the continued gain in diaphyseal length at the proximal end. The middle diaphyseal segment represents that length of the diaphysis that was present at the beginning of the experiment. Although this segment of bone did not increase in length (fig. 3), new bone was added to it during the experiment as indicated by its increase in dry, fat-free weight. Although the proximal diaphyseal segment consisted of entirely new bone, it had the same fluoride concentration (ash basis) as the middle diaphyseal segment.

The uptake of fluoride was greater in the proximal epiphysis than in either the proximal or middle diaphyseal segments. This finding supports the observations of both Volker *et al.* (*4*) and Wallace-Durbin (*3*) that the epiphyses show a greater uptake of radio-fluoride than the diaphyses. These workers have proposed that the uptake of fluoride by bone depends in a large measure upon its vascularity. In the light of the present study this would suggest that the proximal diaphyseal segment is more highly vascular than the middle diaphyseal segment. It is difficult, however, to measure vascularity, and Ham (*14*) has pointed out that the cortex of a long bone is itself highly vascular. It is interesting to note that other radioisotopes such as calcium (*15–17*), sulfur (*18*), strontium (*19*) and phorphorus (*13, 20*) also show a similar distribution, which may change as the animal grows older (*15*).

The fluoride concentration was similar for the proximal and middle diaphyseal segments although their ash contents were significantly different. These findings suggest that with the level and regimen of fluoride administered in the present study, its deposition in various longitudinal segments of the same bone cannot be correlated with the degree of calcification.

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ACUTE AND SUBACUTE TOXICITY STUDIES OF SODIUM FLUORIDE IN ANIMALS

By N. C. Leone, E. F. Geever, and N. C. Moran

Current knowledge of the dosage of fluoride required to produce toxic effects in man is derived principally from suicidal or accidental poisonings (*1–5*). Since the exact doses ingested and absorbed are unknown, interpretation of the relationship between clinicopathological findings and dosage is difficult. The lack of reliable methods for determining minute blood fluoride levels adds to the difficulty of evaluation.

With the more widespread use of fluorides in industry, in agriculture, and in the home, there is need for additional evaluation of acute fluoride effects. Whereas some information is available concerning chronic toxicity, there is less concerning the acute toxic effects in man and animals.

The present study was undertaken to obtain data on the acute and subacute physiological and pathological effects of intravenously and orally administered sodium fluoride in animals. It was also undertaken to determine the safe limits of intravenous administration as an aid to the study of fluoride excretion in man.

METHODS

Two types of animals, dogs and mice, were used in the experiments.

The 27 dogs tested were unanesthetized mongrels of both sexes, weighing from 7 to 10 kilograms. For convenience the dogs were separated into five groups as follows:

Group 1. Five dogs were used for the determination of the acute lethal dose by continuous infusion to the point of death, using a calibrated infusion pump.

Group 2. Nine animals were given sodium fluoride in selected fractions of the acute lethal dose (with the same technique and infusion rate as in group 1) and observed for varying periods.

Group 3. Two dogs were given daily intravenous injections of sodium fluoride for 23 days.

Group 4. Four animals were given sodium fluoride in single doses by mouth.

Group 5. Seven untreated dogs were sacrificed by exsanguination to provide normal control material for the pathological anatomy studies.

Under local anesthesia, the femoral artery and vein of the dogs in groups 1 and 2 were cannulated for recording arterial pressure and for intravenous infusion. Blood pressure was recorded with an Anderson glass capsule manometer (*6*). Respiratory rates and electrocardiograms were recorded by routine methods. In all groups, pupil size and reactivity, tendon reflexes, emesis, defecation, urination, and other significant behavioral and neurological changes in the animals were also observed. In groups 1, 2, and 4, blood was taken before and after administration of sodium fluoride for the determination of serum calcium.

The term "acute lethal dose," as used throughout, refers to that dose at which death occurred as the result of continuous intravenous infusion (group 1). The term "subacute lethal dose" arbitrarily applies to that dose of fluoride causing death one or more hours following administration (group 2).

A sterile, pyrogen-free solution of sodium fluoride (10 mg. of F ion per ml.) was used for intravenous administration; sodium fluoride powder was used for oral administration. All doses are expressed in terms of the fluoride ion.

Necropsy was performed as soon after death as possible, in most instances immediately. Animals that did not die as a direct result of administered fluorides were sacrificed by exsanguination following sodium pentobarbital anesthesia. Necropsy was not performed on the animals that received fluoride by mouth. Tissues were fixed in 10 percent formalin for microscopic examination, and routine methods of tissue preparation, paraffin embedding, and hematoxylin and eosin stains were employed. Special fat stains using oil red O-hemalum and Nile blue were made on renal tissue from 7 controls and 11 fluoride-treated dogs from groups 1, 2 and 3.

Table 2.—*Emetic, cardiotoxic, and lethal doses of sodium fluoride during continuous intravenous infusion of group 1 dogs*

Experiment dog No.	Dose [1] at which—			Terminal ECG event
	Emesis occurred	Arrhythmias appeared	Death occurred	
1	19.8	29.4	36.0	Asystole.
2	20.8	30.7	36.6	Asystole.
3	20.5	33.6	36.8	VF.
4	17.6	28.7	34.6	VF.
Mean	19.7	30.6	36.0	

[1] Doses expressed as milligrams of fluoride ion per kilogram. All animals infused at a rate of 5.4 mg. per minute.

VF denotes ventricular fibrillation.

For mice the oral and intraveneous lethal doses (LD_{50}) were determined by using fasted, male, white mice of uniform weight (10 grams). Each dose level was evaluated in groups of 10 or more mice. The LD_{50} was calculated by the method of Litchfield and Wilcoxon (*7*). The arbitrary end point was 24 hours after administration.

PHYSIOLOGICAL EFFECTS IN DOGS

Group 1

Four dogs were given sodium fluoride by continuous infusion at a rate of 5.4 mg. of fluoride ion per minute to death. The mean acute lethal dose was 36.0± 0.5 mg./kg. (tables 1 and 2). An additional animal was infused at a rate of 1.1 mg. per minute to a lethal dose of 31 mg./kg. but is not included in the

Table 1.—*Summary of fluoride effects in dogs*

Dog group	Dose of fluoride [1] mg./kg.	Rate of intravenous infusion [1] mg./minute	Average body weight (kg.)	Number of dogs	Number of spontaneous deaths	Survival time
Group 1	[2] 36	5.4	8.9	4	4	59–64 minutes.[3]
	31	1.1		1	1	219 minutes.[3]
Group 2	25	5.4	9.3	3	3	1, 18, and 31 hours.
	20	5.4		2	1	36 hours. 7 days (S).
Group 2	15	5.4		4	0	36 hours (S). 7 days (S). 16 days (S, 2 dogs).
Group 3	5	[4] 1	7.9	2	0	23 days (S).

[1] Doses expressed as fluoride ion. [2] Mean of 4 dogs. [3] Death occurred during continuous infusion. [4] Single intravenous injection given daily for 23 days. S=Sacrificed.

calculation of the mean acute lethal dose because of the difference in infusion rate.

No significant change in arterial blood pressure occurred until an average dose of 15 mg./kg. was reached. Beyond this point there was a moderate decrease in pulse pressure. From 20 to 30 mg./kg. there was a moderate fall in systolic and diastolic pressure, after which a rapid, progressive fall of blood pressure occurred (fig. 1).

Little change in mean heart rate was noted at low doses; however, after 20 mg./kg. had been given, there was a progressive decline in rate to zero at death (fig. 1).

Consistent electrocardiographic changes did not appear until a mean dose of 30.6 mg./kg. was reached, when there was conversion in every dog to atrioventricular nodal or ventricular rhythm. The terminal cardiac event was either ventricular fibrillation or asystole (table 2). Occasional changes in the amplitude and direction of the T waves and S–T segments were noted before loss of sinus rhythm but were not consistent. Durations of PR, QRS, and QT intervals did not change until abnormal rhythms occurred. Figure 2 illustrates the electrocardiographic tracings of a representative experiment.

On necropsy the hearts were usually found in systolic contracture. When arrested in diastole, slight mechanical stimulation of the heart induced contracture.

Average respiratory rates did not change appreciably until a dose of about 20 mg./kg. was reached, when an increase occurred. From 30 mg./kg. to the lethal dose, a progressive depression of rate was observed (fig. 1) as also was amplitude. In most of the animals there were frequent short periods of tachypnea, often occurring during the phase of central nervous system depression.

Depression of the central nervous system progressing to coma appeared in all group 1 animals at doses of from 23 to 31 mg./kg. Pupil size and reflex response to light were unaffected until moderately severe central depression developed. At that stage dilation and hypoactive reflexes were noted. Terminally, maximal dilation and areflexia of the pupils occurred. The corneal reflex persisted until the period immediately prior to death. Depression of tendon reflexes paralleled the central depression. Convulsions were not observed in the animals of this group in which the infusion rate was fast. However, in the one case with a slower infusion rate, a stage of neuromuscular hyperexcitability was prominent in the dose range of from 22 to 25 mg./kg. Characteristic of this phase were hyperactive tendon reflexes with clonus, tonic convulsions, and muscle fasciculations. This stage merged into the comatose state described in other animals.

Emesis and defecation were consistent effects with a mean emetic dose of 19.7± 0.8 mg./kg. (table 2). Defecation usually occurred shortly before or immediately after emesis.

Group 2

The dogs in group 2 were infused at the rate of 5.4 mg. per minute. The infusions were stopped at arbitrarily chosen fractions of the acute lethal dose of group 1 (15, 20, 25 mg./kg.), and the animals were observed until death or sacrifice.

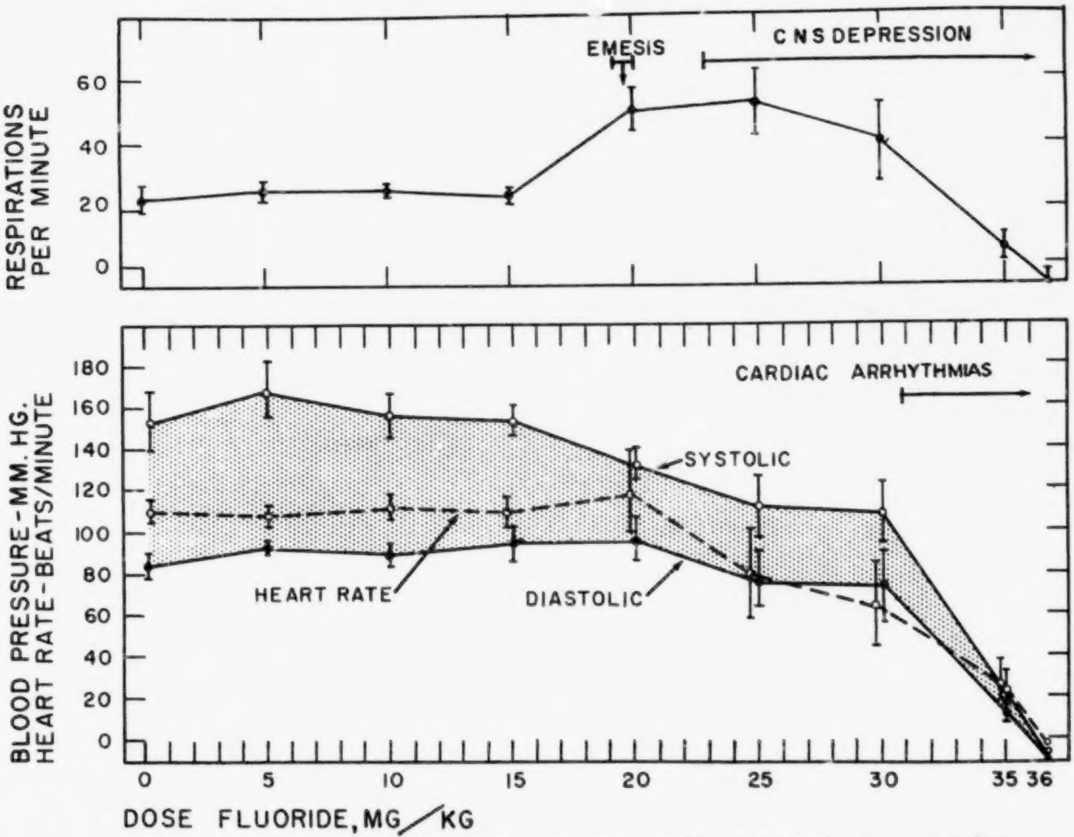
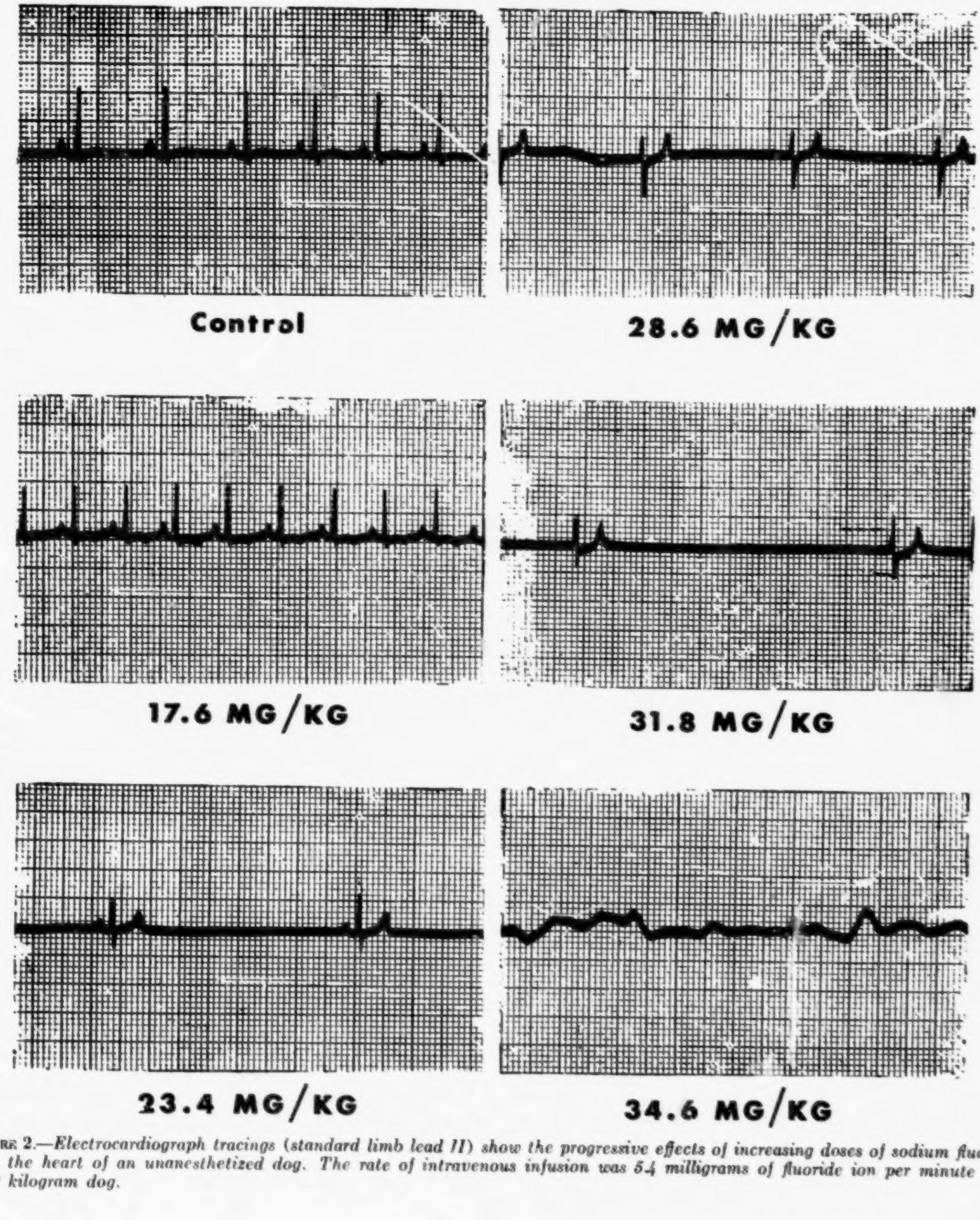


FIGURE 1.—*Respiratory and cardiovascular effects of sodium fluoride administration to group 1 dogs.*

Three dogs which received 25 mg./kg. of fluoride died within 1 to 31 hours after the end of the infusion. One of the two dogs given 20 mg./kg. died at 36 hours, while the other survived until sacrificed at 7 days. None of the four animals administered 15 mg./kg. died as a result of the fluoride. An approximate subacute lethal dose 50 (LD$_{50}$) of 20 mg./kg., therefore, might be assigned to fluoride when given by this method of administration. Table 1 summarizes the results in this group.

Up to the point where the infusions were stopped in the group 2 animals, the cardiovascular and respiratory effects were similar to those of group 1 (fig. 1). There were no consistent electrocardiographic changes in these animals although recordings were not obtained in the period immediately preceding death



Control

28.6 MG/KG

17.6 MG/KG

31.8 MG/KG

23.4 MG/KG

34.6 MG/KG

Figure 2.—*Electrocardiograph tracings (standard limb lead II) show the progressive effects of increasing doses of sodium fluoride on the heart of an unanesthetized dog. The rate of intravenous infusion was 5.4 milligrams of fluoride ion per minute in a 9.2 kilogram dog.*

in those animals which succumbed to the fluoride.

Depression of the central nervous system began before completion of the infusion in all three dogs given 25 mg./kg. and deepened progressively until death. Similar depression was observed in the animal that died as a result of 20 mg./kg. of fluoride. All of the other group 2 animals passed through a short stage of reduced activity, but no severe depression was observed.

Emesis occurred in all of the animals that received 20 or 25 mg./kg. Two of the four dogs receiving 15 mg./kg. of fluoride vomited about 30 minutes after cessation of the infusion.

Group 3

Group 3 consisted of two dogs given fluoride by daily intravenous injections of 5 mg./kg. for 23 days. Blood pressure and respiratory rates were not recorded. The animals remained in good condition with no evidence of toxic effects or weight loss. Electrocardiographs remained normal. The results are summarized in table 1.

Group 4

The four animals in group 4 were each given a single dose of sodium fluoride by mouth in powder form. One received the compound mixed in food and the others in gelatin capsules. Doses of 38, 81, 260, and 3,100 mg./kg. of fluoride were given. The main effects observed were vomiting and frequent defecation. In one case (3,100 mg./kg.) the vomitus was grossly bloody and the animal was mildly stuporous for a short period. In every dog in this group there was apparently complete recovery in 18 to 24 hours.

PHYSIOLOGICAL EFFECTS IN MICE

The oral LD_{50} with standard error was 46.0±1.6 mg./kg. as compared with an intravenous LD_{50} of 23.0±0.9 mg./kg. (table 3). Mice dying within 2 hours after injection showed successively cyanosis, dilatation of ear vessels, depression of respiration, tremors, clonic convulsions, paralysis of the hind legs, loss of righting reflex, depression, respiratory arrest, and death. Those with longer survival periods (2 to 24 hours) went through similar, but less severe, stages, progressing to a long terminal depression.

SERUM CALCIUM AND BLOOD CLOTTING

The effects of fluoride on serum calcium were determined on 11 dogs (table 4). Whole blood was drawn for serum calcium determinations prior to infusion and again immediately before death in the group 1 animals. In the group 2 animals, blood was drawn prior to and at the end of infusion. Blood was drawn from 1 animal in group 4 approximately 1 hour after administration. There was a slight drop in serum calcium in 9 of 11 animals. This finding has been reported by others (*3, 8, 9*).

Although no clotting-time determinations were done, soft, friable, poorly contracted clots were observed in blood samples drawn after fluoride administration.

PATHOLOGICAL ANATOMY OF DOGS

The only gross pathological changes noted in dogs were generalized hyperemia in the animals that died after fluoride administration, whether acute or delayed and, in addition, focal hemorrhages in those dogs that died from 18 to 36 hours after fluoride administration.

TABLE 3.—*Intravenous and oral lethal dose determinations of sodium fluoride in mice*

Dose of fluoride [1] mg./kg.	Ratio of deaths to mice used	Percent deaths
Intravenous [2]		
18	0/10	0
21	3/10	30
24	7/10	70
27	9/10	90
36	10/10	100
48	10/10	100
Oral [3]		
24	0/10	0
36	1/10	10
42	1/10	10
48	3/10	30
54	4/10	40
60	10/10	100
72	10/10	100
84	9/10	90

[1] Expressed as fluoride ion.
[2] Intravenous $LD_{50}=23.0\pm0.9$ mg./kg.
[3] Oral $LD_{50}=46.0\pm1.6$ mg./kg.

Microscopically, all animals that died after fluoride administration and one surviving until sacrificed at 36 hours showed generalized hyperemia and acute focal hemorrhages. All other animals showed some focal hyperemia and focal hemorrhages, but the conditions were no more severe than those in the control group.

Although special attention was given to brain, heart, liver, kidneys, lungs, gastrointestinal tract, and bone marrow, no other histological evidence of cellular injury could be found in any animal. Differences in fat concentration were found, but variations were as great among the controls as among the animals exposed to fluoride. The fat appeared to be liberated from the cytoplasm of the tubular epithelium, particularly along the cortico-medullary junction.

TABLE 4.—*Effects of fluoride on serum calcium in 11 dogs*

Group	Number of dogs	Dose of fluoride [1] mg./kg.	Serum calcium mg./100 cc.	
			Prior to fluoride administration	Immediately after fluoride administration
1	3	[2] 36	8.94 6.51 8.00	7.23 7.75 6.70
2	3	[2] 25	11.15 8.50 8.60	9.65 6.50 7.20
2	2	[2] 20	10.30 9.70	10.30 9.40
2	2	[2] 15	9.20 9.40	8.60 8.20
4	1	[3] 3,100	8.50	7.90

[1] Expressed as fluoride ion.
[2] Intravenous administration.
[3] Oral administration.

Incidental findings, common to the treated and the control dogs, included roundworm and tapeworm infestation of the intestinal tract and microscopic acute and chronic nonspecific inflammation of the lungs, kidneys, liver, and, less often, of the myocardium.

DISCUSSION

On the basis of the present study several points stand out in regard to the acute toxicity of sodium fluoride in dogs.

First, the doses of intravenously administered fluoride required to produce acute toxic effects in animals are high, and there is no evidence of cumulative effects on daily administration of sublethal doses for a period of 3 weeks.

Second, the more uniform effects with a constant rate of intravenous infusion of sodium fluoride are in contrast to the more erratic results and difficulty in producing toxic effects with oral administration.

Third, the physiological effects and pathological changes seen in dogs given toxic doses of fluoride resemble those reported in human fluoride poisoning (*1–4*).

The greater uniformity of results seen in groups 1 and 2 on intravenous administration is most evident in the relatively precise doses at which the various physiological effects occur. In contrast, the erratic results observed when fluoride is given to dogs by mouth are largely due to the loss of undetermined amounts of the administered dose through vomiting.

Diarrhea and bloody stools were observed in some animals in which death was delayed from 1 to 36 hours. Similar findings have been reported in human cases (*1*, *3*, *4*).

The vasodepressor action of fluoride seen in this study has been demonstrated by Greenwood and associates (*10*). It probably is due to a combined depression of the vasomotor centers of the brain and of the vascular smooth muscle. The cardiac arrhythmias, resembling those resulting from high serum potassium, are probably caused by enhanced sensitivity of the myocardium to potassium, secondary to decreased serum calcium concentration. The terminal systolic contracture is of particular interest in view of Loewi's recent observations of a positive inotropic effect of sodium fluoride on the depressed frog heart (*11*). He suggests this action is caused by the formation of a complex of fluoride, a cellular constituent and calcium, and that this complex restores the cellular membrane excitability to normal. However, no conclusive evidence is available to suggest a comparable action in mammalian hearts.

The central nervous system and neuromuscular effects of sodium fluoride may be related to two actions: one, a reduction of ionizable calcium through a fluoride binding effect, resulting in a state of neuromuscular hyperexcitability, that is hypocalcemic tetany, and, second, a depression of the central nervous system as a possible result of enzyme inhibition (*12*).

Routine pathological studies did not demonstrate a specific mechanism of death in experimental fluoride exposure, but at the levels used in this study, and with the short interval to death in many instances, direct toxic cellular effects cannot be ruled out despite the lack of histological evidence of injury. The gross and microscopic pathological examination showed only generalized congestion and acute focal hemorrhages. Similar changes in man and animals have been described for deaths due to fluoride poisoning (*1*, *4*, *13*, *14*,). These changes are interpreted as resulting from injury to vascular endothelium.

Fatty changes have been described in the tubular epithelium of rat kidneys following prolonged administration of sodium fluoride (*15*). In our dogs, shift of renal fat was noted, but no consistent pattern could be found on comparison with the normal controls.

Reviewing the results of this study, it is interesting to compare the findings in animals with human cases of fluoride poisoning (*1–4*). Outstanding is the study of Lidbeck, Hill, and Beeman, who reported a mass accidental poisoning of 263 persons with 47 fatalities (*1*). They described respiratory and cardiovascular depression as a common finding in addition to a number of other manifestations also observed in the present study. An example is: "General collapse developed in most instances but at variable periods of time, apparently depending upon the concentration of the poison. This was characterized by pallor, weakness, absent or thready pulse, shallow, unlabored respiration, weak heart tones, wet cold skin, cyanosis, and equally dilated pupils. When this picture was pronounced, death almost invariably occurred." This description closely follows the findings in our animals receiving a fatal dose of fluoride.

In the present study and the clinical cases described by Lidbeck and associates, convulsions were not regularly observed. However, the carpopedal spasm and spasm of the extremities, described by these authors in a few patients who recovered or where death was delayed, resembled the neuromuscular hyperexcitability seen in one dog in the present study. These effects may be manifestations of hypocalcemic tetany. Generalized convulsions which have been described by others could be on the basis of cerebral anoxia or severe hypocalcemia (*2*).

On a practical basis, the data from this study may be of value in the evaluation and treatment of cases of acute fluoride poisoning in man. For instance, electrocardiographic demonstration of a cardiac arrhythmia in the absence of known heart disease would suggest that about three-quarters of the acute lethal dose had been absorbed and would imply poor prognosis. Central nervous system depression to a marked degree might also denote the absorption of more than half of a lethal dose.

The intravenous lethal dose of fluoride determined in the present study agrees well with those previously reported if differences in injection rates and in species are considered (*10*, *16*, *17*).

This information, establishing the relatively high dosage of fluoride required to produce acute toxic effects in animals, suggests that small intravenous doses may be used with safety for human studies. The absence of toxic effects in dogs given 5 mg./kg. of fluoride intravenously daily for 3 weeks also suggests a considerable margin of safety and an apparent absence of accumulative toxic effects in this period of time.

SUMMARY

In this limited study, designed to evaluate the acute toxic effects of sodium fluoride in dogs and mice, it was demonstrated that:

1. The mean acute lethal dose of sodium fluoride in unanesthetized dogs infused to death by continuous intravenous infusion at the rate of 5.4 mg. of fluoride ion per minute was 36.0±0.5 mg./kg. The principal effects were progressive depression of blood pressure, heart rate, and central nervous system with vomiting and defecation, all occurring with the administration of approximately 20 mg./kg. At a mean dose of 30.6 mg./kg. there was a depression of respiratory rate and a conversion to atrioventricular nodal or ventricular rhythm with terminal ventricular fibrillation or asystole.
2. In a group of dogs infused intravenously with selected fractions of the acute lethal dose, an approximate LD_{50} was estimated to be 20 mg./kg. The major effects observed in this group were vomiting, defecation, and central nervous system depression. In the fatal cases death occurred in 1 to 36 hours.
3. In a group of dogs given 5 mg./kg. intravenous injections daily for 23 consecutive days, no toxic effects were observed.
4. In dogs, single doses up to 3,100 mg./kg. of fluoride by mouth produced only vomiting, defecation, and transient moderate depression.
5. The intravenous LD_{50} in mice was 23.0±0.9 mg./kg. and the oral LD_{50}, 46.0±1.6 mg./kg.
6. A slight drop in serum calcium followed the infusion of fluoride in most dogs in which serum calcium was determined.
7. The pathological findings in those animals which died directly as a result of sodium fluoride administration consisted of generalized hyperemia and acute focal hemorrhages.

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CARIOSTATIC EFFECT AND METABOLISM OF AMMONIUM FLUOSILICATE

I. ZIPKIN AND F. J. MCCLURE

The equal effectiveness of sodium fluosilicate and sodium fluoride in reducing dental caries in the white rat, given at a level of 50 p.p.m. fluorine in drinking water, has been demonstrated by the National Institute of Dental Research, Public Health Service (*1*).

Although sodium fluosilicate, Na_2SiF_6, is cheaper than sodium fluoride, NaF, its use for the fluoridation of municipal water supplies may be limited to some extent by its solubility. Ammonium fluosilicate, $(NH_4)_2SiF_6$, is considerably more soluble than either of these two fluorine compounds, and it is also cheaper than sodium fluoride.

The price (*2*) of the three fluorine compounds and their solubility (*3*) are as follows:

	Price in cents		*Solubility*
	Per lb.	*Per lb. of F*	*Gm. per 100 gm. H_2O at 17.5° C.*
Na_2SiF_6	7.0	10.6	0.65
$(NH_4)_2SiF_6$	11.0	16.0	18.5
NaF	11.5	25.5	4.3

The high solubility and high fluorine content of ammonium fluosilicate make it especially useful in small water plants. It may be used in either liquid or dry chemical feeders. By a special process, it has been made free flowing to prevent clogging of the dry feeder (*4*). The treatment of some water requires the addition of ammonia to reduce the odor and taste, and in this case ammonium fluosilicate may be particularly applicable because it may eliminate the ammoniation operation. It appears, therefore, that ammonium fluosilicate would meet the engineering requirements common to most fluoridation installations as described by Maier (*5, 6*).

In addition, a fluorine compound suitable for fluoridation must meet certain physiological requirements. This study, therefore, presents laboratory data on physiological properties of ammonium fluosilicate pertinent to its use as a practical water fluoridating agent. Sodium fluoride was used as a basis of comparison.

The properties studied in the growing white rat were: (*a*) the inhibition of dental caries; (*b*) development of incisor striations; (*c*) the deposition of fluorine in bones and teeth; (*d*) calcification of bones as determined by ash content; (*e*) average daily weight gain; and (*f*) any gross evidence of untoward physiological effects.

MATERIAL AND METHODS

The sodium fluoride used in this study was an analytical grade reagent and its purity was accepted according to assay. The purity of the ammonium fluosilicate was assessed by analysis for F, Si, and N (Kjeldahl), and the percent of theoretical purity found was 101.7, 96.0, and 100.1, respectively.

Thirty litters of four female weanlings Osborne-Mendel rats were divided into 4 groups of 30 rats each. Group I received distilled water (control); group II received 50 p.p.m. F as NaF; group III received a solution of $(NH_4)_2SiF_6$ containing 50 p.p.m F; and group IV received a composite fluoride solution of the following composition: 0.1105 gm. NaF, 0.3538 gm. $Na_2SiO_3 \cdot 9H_2O$, and 0.0421 gm. $(NH_4)_2CO_3$, made up to 1 liter. The composite solution contained 50 p.p.m. F and furnished a concentration of silicon and ammonium ion equivalent to that provided by the ammonium fluosilicate solution. Use of this solution made it possible to observe the relation of these ions to the effect of fluoride. The pH of all drinking solutions was adjusted to 5.5–6.0. The cariogenic diet (*1*) and all drinking fluids were ingested ad libitum, and fluoride intake was equalized throughout the experiment by substituting distilled water for the fluoride drinking fluid for short periods of time.

At the end of 99 days the surviving animals were killed and their molar teeth scored for dental caries according to Cox and associates (*7*). The degree of incisor fluorosis was arbitrarily scored 0, 1, 2, or 3, that is, none, mild, moderate, and severe, and a mean fluorosis index was calculated for each group. The femurs, mandibles, and molar and incisor teeth of 10 rats of each group, nearest the mean weight for that group, were pooled by tissue, dried, extracted with alcohol and ether, and ground to pass through a 60-mesh sieve. All tissues were then ashed at 550° C. for 3 hours and analyzed for fluorine (*8, 9*).

RESULTS

The caries inhibiting effect of the fluoride solutions and the deposition of fluorine in the bones and teeth are shown in table 1.

There was no gross evidence of toxicity in the rats receiving either ammonium fluosilicate or sodium fluoride, and the average daily weight gains were comparable for each group. In addition, no difference was found in the severity of fluorosis produced in the three groups of experimental rats receiving the different fluoride solutions. The fluorosis indexes for the four groups of rats were 0.0, 1.9, 2.0, and 2.0, respectively (table 1).

Ammonium fluosilicate was as effective as sodium fluoride in inhibiting caries. There was a 28.4 percent and 27.7 percent reduction in the caries incidence and a 52.5 percent and 53.2 percent reduction in the caries score per carious rat in the animals receiving the ammonium fluosilicate and sodium fluoride solutions, respectively.

The chi square test was used to test the difference in the caries incidence between the control and treated rats according to the following formula:

$$\chi^2=\frac{T(AD-BC-T/2)^2}{(A+B)\ (C+D)\ (A+C)\ (B+D)}$$

where T=Total number of rats (90)
A=Number of carious control rats (19)
B=Number of noncarious control rats (3)
C=Number of carious experimental rats (43)
D=Number of noncarious experimental rats (25)

The difference was found to be significant at the 5 percent level using a one-sided significance test ($\chi^2=3.14$).

The caries scores per carious rat, shown in table 1, were compared by analysis of variance (*10*) and the probability (p) values are found in table 2.

For each of the caries categories tested as shown in table 2, the caries results for the experimental groups are significantly lower (table 1) than those for the control group, and no significant differences exist between the groups receiving different fluoride solutions.

TABLE 2.—*Statistical comparison of caries experience: "p" values of differences between groups*

Group	Carious teeth per carious rat	Carious areas per carious rat	Caries score per carious rat
Significance of differences between control and experimental fluorine groups	<0.05	<0.01	<0.01
Significance of differences between experimental fluorine groups	$>.05$	$>.05$	$>.05$

No significant differences are apparent in the deposition of fluorine in the bones and teeth of rats on the various fluoride regimens (table 1). Similarly, no significant differences are found in the ash content of bones and teeth of rats on the various fluoride regimens. These current data on caries inhibition, fluorine deposition, and ash agree closely

TABLE 1.—*Daily gain in weight, caries inhibition, deposition of fluorine, and percent ash in bones and teeth of rats receiving distilled water and different fluoride solutions*

Item	H_2O	NaF	$(NH_4)_2SiF_6$	$NaF+$ Na_2SiO_3+ $(NH_4)_2CO_3$
Number of rats	22	24	21	23
F intake (mg.)	0	60.7	59.8	59.9
Days on experiment	99	99	99	99
Initial weight (gm.)	50	50	51	49
Final weight (gm.)	190	190	180	185
Average daily gain (gm.)	1.4	1.4	1.3	1.4
	Caries diagnosis			
Number rats with caries	19	15	13	15
Percent rats with caries	86.4	62.5	61.9	65.2
Number carious teeth per carious rat	3.0	2.3	2.4	2.3
Number carious areas per carious rat	6.2	3.1	3.2	2.8
Caries score per carious rat	14.1	6.6	6.7	5.6
Fluorosis index	0	1.9	2.0	2.0
	Percent fluorine in bones and teeth (ash basis)			
Molars	.006	.216	.226	.23
Incisors	.002	.168	.180	.16
Mandibles	.005	.419	.439	.43
Femurs	.005	.452	.462	.47
	Percent ash in bones and teeth			
Molars	75.8	76.1	75.6	75.3
Incisors	76.1	76.0	76.5	75.7
Mandibles	70.0	71.3	70.7	71.1
Femurs	63.9	63.1	64.3	64.8

with similar data obtained in a previous study (*1*).

Hence, it appears that ammonium fluosilicate and sodium fluoride were similar with respect to all the criteria investigated. There were no significant differences in the average daily weight gains, the degree of fluorosis, the reduction of dental caries, and in the amounts of fluorine and ash deposited in the bones and teeth.

DISCUSSION

It is not surprising that ammonium fluosilicate proved to be similar to sodium fluoride at a level of 50 p.p.m. F in drinking water in its cariostatic effect and in its ability to deposit fluorine in bones and teeth of the white rat. Previous comparisons between sodium fluoride and sodium fluosilicate at 50 p.p.m. F in drinking water have shown no differences in the amount of fluorine deposited in bones and teeth (*8*), in the extent of incisor fluorosis, and in the reduction of caries (*1*).

Theoretically, ammonium fluosilicate would yield fluorine ion quantitatively upon hydrolysis according to the following scheme (*11*):

$$\begin{array}{c} \mathrm{(NH_4)_2\,SiF_6 \rightleftharpoons 2NH_4^+ + SiF_6^=} \\ \underline{\mathrm{SiF_6^= + 2HOH \rightarrow SiO_2 + 6F^- + 4H^+}} \\ \mathrm{(NH_4)_2SiF_6 + 2HOH \rightarrow 2NH_4^+ + 6F^- + 4H^+ + SiO_2} \end{array}$$

These reactions also indicate that solutions of ammonium fluosilicate would have an acid reaction and in fact did have a pH of 3.4–3.6.

In an acid medium, such as the gastric juice, the silicon of Na_2SiO_3 would be present as SiO_2 and hence should react like the silicon of $SiF_6^=$ according to the following reactions:

$$\begin{array}{c} \mathrm{Na_2\,SiO_3 \rightleftharpoons 2Na^+ + SiO_3^=} \\ \underline{\mathrm{SiO_3^= + 2H^+ \rightarrow SiO_2 + H_2O}} \\ \mathrm{Na_2SiO_3 + 2H^+ \rightarrow 2Na^+ + SiO_2 + H_2O} \end{array}$$

The composite solution was similar to the ammonium fluosilicate and sodium fluoride solutions according to the criteria investigated. It appears, also, that neither the ammonium ion nor silicon interfered with the physiological reactions characteristic of the fluoride ion.

The results of this study indicate, therefore, that $(NH_4)_2SiF_6$ is hydrolyzed in dilute solution and behaves physiologically like the solution of NaF.

CONCLUSIONS

1. A comparison was made of the ability of sodium fluoride, NaF, and ammonium fluosilicate, $(NH_4)_2SiF_6$, solutions containing 50 p.p.m. F to reduce caries and deposit fluorine in the bones and teeth of the white rat.
2. No differences were observed in the amount of fluorine and ash deposited in the molars, incisors, mandibles, and femurs.
3. There was no difference in the rate of growth among all groups of rats.
4. There was no difference in the production of incisor striations.
5. Ammonium fluosilicate was as effective as sodium fluoride in inhibiting caries in the white rat.
6. The data suggest that ammonium fluosilicate may be equally as effective as sodium fluoride as a fluoride carrier for the fluoridation of municipal water supplies.

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DISTRIBUTION AND EXCRETION OF HEXAFLUOPHOSPHATE

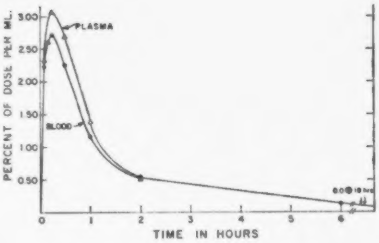
R. C. Likins, I. Zipkin, and H. G. McCann

In a recent study of the metabolism of various complex fluorides (*1*), it was shown that administration of potassium hexafluophosphate to rats resulted in no increase in fluoride content of the skeletal and dental tissues, and had no effect on either incidence or severity of dental caries. These findings, together with evidence attesting to the low toxicity of hexafluophosphate in both the frog and rabbit (*2*), indicate that this anion is relatively, if not completely, inert. While at least one other metabolically inactive complex inorganic fluoride (KBF_4) has been reported (*3*), the physiological properties of PF_6^- are of some theoretical interest in that it represents, to our knowledge, the only phosphorus-containing anion which is not readily metabolized. PF_6^- is also unique in that it is the only compound of pentavalent phosphorus known to exist as the free anion (*4*). It was decided, therefore, to extend information relative to its metabolism by investigating the occurrence of PF_6 in tissues other than those of bone and teeth, and to determine the mode of its excretion.

Experimental: Preparation of labelled hexafluophosphate.—$KP^{32}F_6$ was synthesized according to Woyski (*5*) using PCl_5 prepared from radioactive phosphorus (*6, 7*):

$KCl + P^{32}Cl_5 + 6HF \rightarrow KP^{32}F_6 + 6HCl$ (in liquid HF). After recrystallization from hot water, the percentage composition of the product was: P, 16.75; F, 61.57; free F ion, 0.18. Theoretical for KPF_6: P, 16.83; F, 61.93. Radioactivity was determined by conventional procedures using an end-window counter, and the values obtained were corrected for self absorption. Fluoride was determined according to Willard and Winter (*8*) as modified by McClure (*9*). Neutron irridation of the commercially available ammonium salt (Ozark-Mahoning Co., Tulsa, Okla.) may offer a more feasible method of preparing radiophosphorus-tagged PF_6^-.

Collection of excreta. Metabolism cages were constructed to permit separate collection of urine and feces. A series of holes 5½-inch in diameter were cut in a wood board, and upholsterers' tacks inserted on half-inch centers around the perimeter of each. Using the tacks as a frame, nylon cord was strung to form a screen floor beneath each opening. Rats have been retained up to one week in this type of cage. For retention over longer periods of time, stainless-steel wire may be substituted for the nylon cord. An inverted 2 liter beaker provided with a three-eighth-inch hole to admit a drinking tube served as a cage. For stability, scotch tape was placed across the top of the water bottle and fastened to the board on either side. A strip of filter paper was attached to the back of the board, angulated approximately 45° to horizontal, and fastened to the edge of the work bench. The free edge was folded back to form a collection trough for the feces. Urine was trapped by absorption on the paper. Female Sprague-Dawley rats weighing 100-120 g were employed throughout. Twenty-seven rats were injected intraperitoneally with 1 mg. of $KP^{32}F_6$ 1 μc/mg. in 1 ml. of distilled water, and placed in separate metabolism cages for 1, 3, 6, 12, or 24 hours. At the end of the collection periods each rat was induced to empty its bladder by digital pressure applied at the base of the tail. The filter paper was placed under ultraviolet light and the strongly fluorescing urine spots cut out. These were combined with the nylon cord and both extracted with hot distilled water. Recovery of labelled phosphorus added as PF_6^- to 3 control urine papers was 97.0±1.3% (mean ± S.E.). An additional 42 rats were injected with 1 mg. of $KP^{32}F_6$ as before and sacrificed by decapitation at the end of 5, 10, 15, and 30 minutes, and 1, 2, 6 and 18 hours. The animals were bled individually into oxalated beakers and the whole blood and plasma analyzed for radiophosphorus. Two rats were injected intravenously with 1 mg. of $KP^{32}F_6$ dissolved in 0.2 ml. of physiological saline, and sacrificed by decapitation at the end of 10 minutes. The heart, liver, lungs, kidneys, spleen, and a portion of thigh muscle were excised, blotted, and weighed. The samples were ignited for 3 hours at 550° C. dissolved in 1N HCl and analyzed for radiophosphorus. Recovery of labelled phosphorus added as PF_6^- to 6 control tissue samples was 95.9±1.6% (mean ± S.E.).



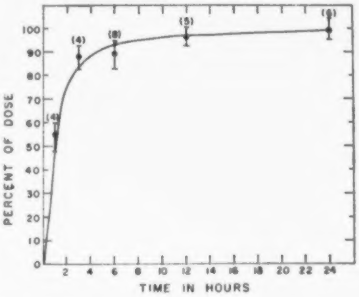


FIGURE 1 (top).—*Conc. of radiophosphorus in blood and plasma following intraper. inj. of* $KP^{32}F_6$.

FIGURE 2 (bottom).—*Excretion of radiophosphorus in urine following intraper. inj. of* $KP^{32}F_6$.

Results.—Concentration of radioactivity in whole blood and plasma reached a maximum at 15 minutes, then fell precipitously (fig. 1). No detectable activity was found in the blood 18 hours after injection. Concentration values were consistently higher in plasma than in blood, and may reflect differences in water content. The findings are consistent with the belief that blood cells are freely permeable to the PF_6^- ion.

Ten minutes after intravenous injection the mean concentration of radiophosphorus in the tissues, expressed as percent of dose per gram of fresh weight, was: liver, 0.57; spleen, 0.55; heart, 0.51; lungs, 0.97; kidney, 1.67; and muscle, 0.29.

The high radiophosphorus content of kidney seems in accord with the role of this organ in the excretion of the label. Thus 55 percent of the dose had been cleared at the end of one hour, and 88 percent by the end of 3 hours (fig. 2). The 24-hour urine contained all of the injected radiophosphorus. The rapidity of clearance suggests that tubular reabsorption was minimal, if not absent.

In 8 urine samples selected at random, the ratio of calculated-to-found fluoride was 0.98±0.03 (mean±S.E.). This finding substantiates the physiological stability of PF_6^-. Similarly, the quantitative recovery of injected radiophosphorus in urine can only be explained by the inability of the rat to hydrolyze PF_6^- to $PO_4^{\equiv}$ and F^-.

The physiological stability of this ion, coupled with its quantitative excretion in the urine, suggests consideration of radiophosphorus-tagged hexafluophosphate as a possible tool in renal function studies.

Summary.—1. Distribution and excretion of radiophosphorus labelled hexafluophosphate ion has been investigated in the rat. 2. The concentration of PF_6^- in whole blood and plasma reached a maximum at 15 minutes, fell precipitously, and none was found 18 hours after its injection. 3. Evidence is presented that PF_6^- is not hydrolyzed by the rat. 4. Injected PF_6^- is rapidly and quantitatively excreted in the urine.

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ABSORPTION OF VARIOUS FLUORINE COMPOUNDS FROM THE GASTROINTESTINAL TRACT OF THE RAT

I. ZIPKIN AND R. C. LIKINS

ABSTRACT

Zipkin, I. and R. C. Likins. *Absorption of various fluorine compounds from the gastrointestinal tract of the rat.* Am. J. Physiol. 191(3): 549–550. 1957. The absorption of variously bonded fluorine compounds from the gastrointestinal tract of the rat was determined. NaF, Na_2SiF_6, Na_2PO_3F and SnF_2 were absorbed to the same extent (48%), whereas KPF_6, $EtNPF_6$ and KBF_4 were absorbed to a significantly greater degree (74%). The results suggest that covalently held fluorine compounds are absorbed at a greater rate than the fluoride ion.

Recent studies have indicated that NaF, Na_2SiF_6, Na_2PO_3F and SnF_2 are similar in their ability to inhibit caries and to be deposited in the bones and teeth (*1–4*). However, it has also been shown that KPF_6 is not hydrolyzed in the rat (*2*, *4a*), and that $NaBF_4$ is similarly inert in the human being (*5*).

In view of the physiological similarity of NaF, Na_2SiF_6, Na_2PO_3F and SnF_2, it was the purpose of the present study to compare their rates of absorption. Although it appears that absorption of KPF_6 (*2*) and $NaBF_4$ (*5*) from the gastrointestinal tract is not the determining factor in their unavailability, it was of interest to compare the rates of absorption of these two compounds with those of the simple fluorides listed above. The rate of absorption of Et_4NPF_6 was compared to that of KPF_6 to determine the effect of the cation.

In a preliminary study, rats were intubated with 2.0 ml. of a solution of NaF containing 100 μg F/ml. They were then killed immediately and at 5, 15, 30, 60 and 90 minutes thereafter. The entire gastrointestinal tract was removed, 10.0 ml. of a low-F $Ca(OH)_2$ suspension containing 5 gm./100 ml. were added (*6*) and the samples were ashed slowly (7) and analyzed for fluoride by conventional procedure (*8*, *9*). The percentage of administered fluoride which had passed through the gastrointestinal tract and therefore had been absorbed (the gastrointestinal tract was clear of fecal material, and hence no fluoride was lost by way of the feces) was 12, 22, 36, 50, 72 and 86 percent, respectively, for each of the above time intervals. Fifty percent of the administered NaF was absorbed at the end of 30 minutes, and this interval of time was taken for killing of all rats. Groups of 10 male Holtzman rats weighing 110–120 gm. were fasted for 72 hours and then intubated with 2.0 ml. of each of the seven compounds containing 100 μg F/ml. of solution. The results are shown in table 1.

Compound	% of Fluorine Intake Absorbed*	Compound	% of Fluorine Intake Absorbed*
1. NaF	40.6±4.3	5. KPF_6	77.2±3.2
2. Na_2SiF_6	50.9±3.1	6. Et_4NPF_6	67.7±3.7
3. Na_2PO_3F	43.2±2.8	7. KBF_4	75.8±2.7
4. SnF_2†	50.0±1.7		
Mean	48.4±1.6	Mean	73.6±2.0

* Mean ± SE based on 10 rats/compound.

† Prepared immediately prior to use.

TABLE 1.—*Absorption of various fluorine compounds from the gastrointestinal tract of the rat*

NaF, Na_2SiF_6 and KBF_4 (used instead of $NaBF_4$) were commercially available reagents of high purity. SnF_2 was obtained from Dr. J. C. Muhler, University of Indiana Dental School, Bloomington, Ind. Na_2PO_3F, KPF_6 and Et_4NPF_6 were obtained from the Ozark-Mahoning Co., Tulsa, Okla. Quantitative recoveries of fluoride from all fluorine compounds were obtained when a 2.0-ml. solution of each compound containing 200 μg F was added to the isolated gastrointestinal tract.

It is apparent that these seven fluorine compounds fall into two distinct groups as shown in table 1. NaF, Na_2SiF_6, Na_2PO_3F and SnF_2 are absorbed to the same extent (according to analysis of variance F=1.27, $P < 0.05$ for compounds 1–4; F=2.10, $P < 0.05$ for compounds 5–7) thus presenting further evidence for their metabolic similarity. The similarity in rates of absorption of NaF and Na_2SiF_6 provides additional support for the equal applicability of these compounds for water fluoridation. KPF_6 and KBF_4, which have been reported to be metabolically inert (*2*, *5*), have a rate of absorption significantly higher ($P < 0.001$) than that of the first group (for the difference of the means for the rate of absorption of these two groups t=9.60, $P < 0.001$). It is apparent that the rate of absorption of KPF_6 and Et_4NPF_6 is not affected by their cations. The difference in rate of absorption of the two groups of fluorine compounds is probably related, to a large extent, to their dissimilarity in electronic structure. In the former group, fluorine is electrovalently bound and is present as the fluoride ion, whereas in KPF_6 and KBF_4, fluorine is covalently held and hence has lost its identity as the fluoride ion. It is of interest to note in this study that the rate of absorption of the physiologically inert complex fluoride compounds from the gastrointestinal tract of the rat significantly exceeds that of the physiologically active simple fluorides.

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THE EFFECT OF FLUORIDE INGESTION ON THE COMPOSITION AND SOLUBILITY OF MINERALIZED TISSUES OF THE RAT

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The formation of fluorapatite in aqueous solution has been noted by MacIntire, Hardin, Oldham, and Hammond *(1)* in studies of the cause of phosphate insolubility, by Montel and Chaudron *(2)*, Rathje *(3)*, in their studies on the synthesis of various apatites, and by McCann *(4)* in a study of the reactions of fluoride with hydroxyapatite. Neuman, Neuman, Main, O'Leary, and Smith *(5)* have shown that fluorapatite is formed below the solubility product of calcium fluoride by ionic exchange. McCann and Bullock (6) indicated that a number of reactions occur between fluoride ion and powdered enamel and dentin, but the principal one, up to a concentration of about 100 p.p.m. F^-, is the formation of fluorapatite. Gerould *(7)*, Syrrist *(8)*, and Rathje *(9)* have presented evidence showing that it is quite probable that the principal reaction occurring between fluoride and the hard tissues of the body in vivo is the formation of fluorapatite.

It was the purpose of this study to obtain additional evidence of the formation of fluorapatite in vivo in skeletal and dental tissues. Information was obtained also on the properties of tissues fluorosed in vivo as well as in vitro, as shown by solubility and ion-exchange studies. A chemical analysis of control and fluorosed tissues furnished additional data on the effect of fluoride on tissue composition.

EXPERIMENTAL

Litters totaling 235 weanling Sprague-Dawley rats, weighing between 36 and 47 grams, were divided between 2 groups, each group receiving half of each litter. Both groups were fed an adequate diet low in fluoride (1 p.p.m.) composed of cornstarch, 66.5 percent; whole milk powder, 27.0 percent; yeast, 5.0 percent; cod liver oil, 1.0 percent; salt mixture (500 gm. NaCl, 53.2 gm. ferric citrate trihydrate, and 3.9 gm. copper sulfate pentahydrate), 0.5 percent. One group received 100 p.p.m. fluoride as NaF in the drinking water; the other group served as controls and received distilled water. The average body weight after 100 days on experiment was 176.2 gm. for the 118 controls and 157.8 gm. for the 117 fluoride-treated rats. After sacrifice the animals were autoclaved and the femora, mandibles, humeri, molars, and incisors removed and pooled by groups. Fat was extracted with alcohol and ether, and the samples dried, powdered to pass a 60-mesh sieve, and the enamel and dentin of the molars and incisors separated by the method of Manly and Hodge (10).

Analytical Methods.—Samples were analyzed in duplicate or triplicate for calcium, phosphorus, magnesium, fluoride, sodium, and carbon dioxide as described previously *(6)* with a precision of 0.15, 0.2, 4, 3, 2, and 1 percent respectively. (Calculated as coefficient of variation *(12)*.) Some of the solubility tests described below were made by titrating the combined calcium and magnesium present in the filtered solutions with the sodium salt of ethylenediamine tetraacetic acid using Erichrome Black T as indicator *(11)*.

Solubility Studies.—The solubility of each tissue was determined by adding 0.250 gm. of solid to 1 liter of water in a polyethylene bottle and shaking occasionally for a period of 30 days. The temperature was maintained at 37° C. by storage in a constant temperature room. The filtrate was then analyzed for calcium and phosphorus. Attempts to determine the magnesium and fluorine were unsuccessful due to the low concentration of these components. The comparative solubilities of several of the samples in lactic, acetic, and hydrochloric acids were obtained by shaking 0.050 gm. of solid in 25 ml. of solution for 48 hours in a mechanical shaker at room temperature (about 30° C.). Solubility was then determined by titrating the combined magnesium and calcium as described above.

Rate of Fluoride Uptake in Vitro.—The rate of the in vitro reaction between fluoride ions and control tissue was determined at 37° C. by adding 0.1 gm. of the solid to 800 ml. of solution containing the desired concentration of fluoride as NaF. The samples were stirred constantly for a specified length of time, then filtered through sintered glass, and the solid analyzed for fluorine.

Exchange Studies With KOH.—One to 2 tenths gram samples of this solid were added to 50 ml. of 1 N KOH maintained at 37° C. for a period of 1 to 7 days with occasional shaking. Samples were then filtered, the paper ignited, and both liquid and solid analyzed for fluorine.

RESULTS

Analysis of Tissues (table I).—The amount of fluoride deposited is a function of the kind of tissue involved. The three different bones have about the same percent of fluoride, the molar and incisor dentin have a lower content, and the molar and incisor enamel lower yet. The samples of molar and incisor enamel, in contrast to the bone and dentin samples, differ somewhat from each other, probably because of a difference in the way fluoride is deposited in enamel as compared with that in dentin and bone.

It is interesting to note that the bones from the fluoride-treated rats contain less CO_2 than the controls, whereas the CO_2 content of the molar dentin remains practically constant. The controls vary from 2.57 to 3.01 percent; tissues from the fluoride-treated rats range only from 2.61 to 2.66, which is constant within the absolute experimental error of ± 0.03 percent. However, the CO_2 content of

TABLE I.—*Analytical data on dry, fat-extracted rat tissues*

SAMPLE	Ca (%)	P (%)	Ca:P (RATIO)	F (%)	Mg (%)	Na (%)	CO_2 (%)	ASH (%)
Femora—Control	24.38	12.28	1.536	0.010	0.60	0.50	2.76	67.3
Femora—Fluoride	24.70	12.25	1.560	0.832	0.74	0.53	2.64	69.0
Humeri—Control	24.51	12.13	1.564	0.013	0.65	0.50	2.87	67.9
Humeri—Fluoride	25.23	12.48	1.565	0.850	0.74	0.55	2.66	70.5
Mandibles—Control	26.56	13.28	1.547	0.012	0.64	0.53	3.01	73.6
Mandibles—Fluoride	26.28	12.96	1.568	0.822	0.72	0.63	2.63	73.5
Molar dentin—Control	28.03	14.04	1.545	0.009	0.37	0.58	2.57	77.2
Molar dentin—Fluoride	28.56	14.10	1.567	0.480	0.44	0.70	2.61	78.2
Incisor dentin—Control	26.80	15.41	1.345	0.004	1.49	---	1.92	79.5
Incisor dentin—Fluoride	27.56	15.26	1.397	0.521	1.92	0.76	1.97	80.8
Molar enamel—Control	35.32	17.43	1.568	0.004	0.32	0.73	---	---
Molar enamel—Fluoride	34.86	17.35	1.555	0.077	0.37	0.79	---	---
Incisor enamel—Control	34.74	17.35	1.550	0.002	0.25	0.66	---	---
Incisor enamel—Fluoride	33.26	16.90	1.522	0.197	0.66	0.81	---	---

Table II.—*Composition of tissues on ash basis*

SAMPLE	Ca (%)	P (%)	F (%)	Mg (%)	CO_2 (%)
Femora—Control	36.22	18.25	0.02	0.89	4.10
Femora—Fluoride	35.80	17.76	1.21	1.07	3.83
Humeri—Control	36.10	17.86	0.02	0.95	4.23
Humeri—Fluoride	35.78	17.69	1.21	1.05	3.77
Mandibles—Control	36.09	18.04	0.02	0.87	4.09
Mandibles—Fluoride	35.75	17.63	1.12	0.98	3.58
Molar dentin—Control	36.32	18.18	0.01	0.48	3.33
Molar dentin—Fluoride	36.52	18.03	0.61	0.56	3.34
Incisor dentin—Control	33.71	19.39	0.05	1.88	2.42
Incisor dentin—Fluoride	34.12	18.89	0.64	2.38	2.42

the incisor dentin of both control and fluoride-treated rats is considerably different, which is true of its magnesium content as well (1.49 and 1.92 percent vs. about 0.4 percent for molar dentin).

In every case the magnesium content is higher in the fluoride-treated animals than in the controls. Particularly striking is the increase in magnesium content of the incisors of the rats which received fluoride, especially since the incisors were completely renewed during the experiment, and all the magnesium found on analysis (present at sacrifice) had been incorporated during the test period.

Composition of Tissues on the Ash Basis (table II).—In each of the bones the incorporation of fluoride has not increased the calcium content of the ash. There is in fact a slight decrease in calcium, although the calcium to phosphorus ratio increased slightly as a result of a somewhat larger reduction in phosphorus content. In the dentin there is a slight increase in the percent of calcium. However, the effect on the ash of the bone appears to be negligible. The magnesium in the ash of all tissue was increased by fluoride.

Solubility Tests (tables III and IV).—A considerable reduction in water solubility after in vivo fluoride exposure occurred with most of these tissues as seen in table III. The reason for the difference between calcium versus phosphorus solubility in the molar dentin compared to that of the other tissues is not known. The difference in the Ca:P ratio of the solution of incisor dentin as compared with the other tissues may be accounted for by the difference in the Ca:P ratio of the solid (table I). In the case of molar enamel the decrease in solubility was slight, possibly on account of the small amount of fluoride incorporated. Further solubility studies were carried out using lactic, acetic, and hydrochloric acids of various strengths as seen in table IV. In each case the solubility of the tissues from the fluoride-treated rats was considerably less than that of the corresponding controls. The solubility was then compared with samples of control tissues treated in vitro with fluoride ion at a concentration of 2 p.p.m. for a period of 2 months at 37° C. with occasional shaking. This material had a fluorine content somewhat higher than the material obtained from the rats in vivo (femora, 1.13 per cent vs. 0.83 percent; incisor dentin, 0.87 percent vs. 0.52 percent; molar dentin, 0.85 percent vs. 0.48 percent), and was assumed to contain most of its fluorine as fluorapatite as shown by previous work *(6)*. These tissues gave solubilities in several acidic solvents close to the in vivo-treated material in the case of the femora and incisor dentin. The molar dentin treated controls were somewhat less soluble than the in vivo fluoride samples (table IV).

Another type of in vitro study was then carried out on the in vivo fluoride-treated tissues. It was thought that if the fluoride were present in the tissues as fluorapatite it would exchange with hydroxyl ion (as supplied in 1 N KOH). However, a test of these tissues showed that very little of the fluoride could be exchanged (about 5 percent in 3 hours, 15 to 18 percent in 1 to 7 days). Megirian *(13)* failed to desorb any fluoride from glycol-ashed bone previously treated with NaF solution for 4 days. The hydroxyl ion concentration used by Megirian was much lower than that employed here, in addition to the fact that the tissue had been ashed. The same procedure (treatment with KOH) was also carried out on control material that had been treated for a short time with fluoride in vitro, and it was found that most of the fluorine could be exchanged. A more thorough study was then made, the results of which are shown in figure 1. Thus, for example, in the case of the femora it can be seen that in 1 day's exposure to 2 p.p.m. F^- at 37° C., the fluoride content is increased to 0.21 percent. Of this, 68 percent can be removed by 1 N KOH in 1 to 7 days. In 1 week the fluoride increases to 0.63 percent of which 34 percent can be removed by treatment with KOH.

The speed of reaching equilibrium in the reaction of fluoride ion with powdered bone (femur) and dentin is seen to be a relatively slow process, while the reaction of fluoride (ions) with enamel, although quantitatively limited, reaches equilibrium rapidly (fig. 1). When an attempt is made to exchange the fluoride from in vitro-treated tissues with hydroxyl ions, the difference in the reaction of bone and dentin as compared with enamel is again evident, that is, most of the fluoride can be exchanged from the enamel very quickly regardless of time of fluoride treatment; however, the fluoride can be removed from dentin and bone only if the material had been treated for a very brief time with fluoride ion. As the time of fluoride treatment increases the percent that can be removed by KOH treatment decreases. Apparently the fluoride is only on the outer surfaces of the crystals on short exposure and penetrates deeper with time.

Table III.—*Solubility of tissues in water*

SAMPLE	Ca (MG./l)	P (MG./l)	Ca:P (RATIO)
Femora—Control	13.60	7.55	1.394
Femora—Fluoride	9.08	5.50	1.276
Humeri—Control	13.65	7.64	1.382
Humeri—Fluoride	8.38	5.50	1.178
Mandibles—Control	13.60	6.45	1.629
Mandibles—Fluoride	10.55	5.14	1.587
Molar dentin—Control	20.7	7.05	1.227
Molar dentin—Fluoride	10.11	5.72	1.367
Incisor dentin—Control	9.55	9.33	0.792
Incisor dentin—Fluoride	7.08	6.85	0.800
Molar enamel—Control	5.90	3.00	1.521
Molar enamel—Fluoride	5.55	2.80	1.533
Incisor enamel—Control	7.00	4.50	1.202
Incisor enamel—Fluoride	4.20	2.35	1.382

DISCUSSION

The analysis of hard tissues of rats receiving 100 p.p.m. fluoride in drinking water as compared with tissues from control rats, has indicated that this amount of fluoride has little effect on the calcium and phosphorus content but does have an effect on the carbon dioxide and magnesium content. The results on CO_2 and magnesium are in agreement with the work of Kick et al. *(14)*, rather than that of Wadhwani *(15)* which was in direct contrast to that of

TABLE IV.—*Comparative solubilities**

SAMPLE	WATER	LACTIC ACID			ACETIC ACID			HYDROCHLORIC ACID		
		.00001 N	.0001 N	.001 N	.00001 N	.0001 N	.001 N	.00001 N	.0001 N	.001 N
Femora										
Control	18.2	22.2	21.9	29.1	22.9	18.2	21.2	22.5	26.2	58.3
Fluoride	13.9	13.9	14.2	15.9	14.2	15.9	16.9	15.9	15.9	45.0
F-treated control	13.3	14.2	14.2	16.6	14.2	14.6	15.9	13.6	15.3	42.2
Incisor Dentin										
Control	28.8	28.8	30.8	39.4	26.8	25.8	37.4	26.5	30.1	67.2
Fluoride	13.6	14.2	18.2	25.1	13.6	17.5	25.2	13.9	19.9	47.6
F-treated control	13.9	11.1	13.0	26.8	13.9	14.8	25.0	16.7	18.5	59.2
Molar Dentin										
Control	25.0	30.0	30.9	40.9	25.0	30.9	38.9	30.5	32.6	71.8
Fluoride	14.8	17.6	24.1	31.5	17.6	17.6	31.8	18.5	29.7	59.2
F-treated control	11.1	13.9	14.8	23.1	13.0	14.8	23.1	15.7	20.4	59.2

*In milligrams Ca + Mg per liter.

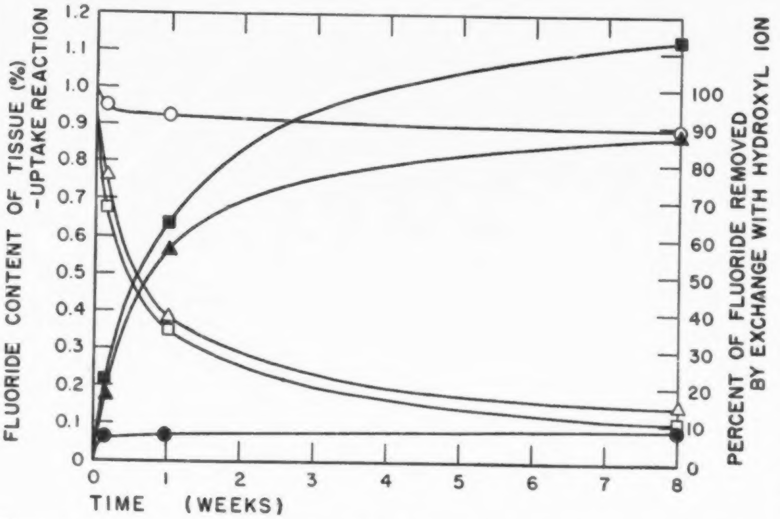

FIGURE 1.—*Rates of in vitro reaction of hard tissues showing fluoride uptake (■, ▲, ●), and amount of this fluoride exchanged by 1 N KOH solution (□, △, ○). The dentin curves represent both molar and incisor dentin which gave almost identical results. Femur is denoted ■, □; dentin ▲, △; and enamel ●, ○.*

earlier workers. Previous work by Hendricks *(16)* and McCann and Bullock *(6)* has indicated that magnesium is present in calcified tissues, not as an integral part of the crystal, but bound on the surface by adsorption. Possibly the incorporation of fluoride in the tissues caused larger amounts of magnesium to be adsorbed because of the affinity of fluorine for magnesium. Apparently calcium, being an integral part of the crystal, is not affected in the same manner.

There is a notable similarity between the products of fluoride acting in vivo and the products formed by fluoride acting in vitro, i.e., by allowing the control tissues to react with 2 p.p.m. fluoride for as long as 8 weeks. This latter reaction in vitro is known to form fluorapatite *(6)*. A similarity in behavior of the in vitro versus in vivo fluorosed tissues is exhibited in the solubility studies. Likewise, in the exchange studies with KOH, there is a difference in behavior shown by the samples treated in vitro with fluoride for a few hours or days versus a treatment for 8 weeks. The products of this longer treatment period in vitro become increasingly similar to the reaction products formed in vivo. Evidently with the increasing reaction time in vitro fluoride penetrates further into the crystals and its reaction becomes more and more similar to the reaction which occurs in vivo. This evidence seems to support the formation of fluorapatite in the tissues which were fluorosed in vivo.

Equilibrium rate studies with fluoride ions showed that for dentin and bone the attainment of equilibrium is a slow process. The most probable explanation for this is that the reaction is taking place on the surface by ionic exchange *(6)*, but in order to proceed further, recrystallization must take place probably by the process of thermal aging, as shown by Weikel and Neuman *(17)* and Neuman and Mulryan *(18, 19)* in the case of phosphate exchange in unashed bone. Since the enamel crystals are larger than those of dentin and bone *(20)*, recrystallization apparently takes place too slowly to be detected in a period of 2 months. The small amount of organic material as compared with dentin and bone may also be a factor. An apparent equilibrium therefore is observed in a few hours. Bone and dentin on the other hand appear to permit a relatively rapid recrystallization so that the reaction with fluoride continues at a noticeable rate for 60 days and perhaps longer. The formation of fluorapatite apparently takes place by ionic exchange through a process of recrystallization into the crystal after surface positions are exchanged.

It has been pointed out by Hendricks *(16)*, and has been shown in previous work with powdered enamel and dentin *(6)*, that enamel has hidden surfaces that are not reached unless the crystals are ground extremely fine, or, of course, are actually dissolved by severe attack as with acid. Substances that are incorporated during formation are trapped on these surfaces and are not released except by an extremely slow reaction, when made accessible by some treatment. It is believed that bone and dentin can pick up considerable amounts of fluoride by adsorption into the tissues through ionic exchange and diffusion after the tissue has been completely formed, as well as by incorporation during growth. However, if enamel can pick up only a very limited amount of fluoride on its outer surface, the greater part of its fluoride content must be incorporated during growth. The rat incisor tissue was completely renewed during the experiment, whereas the molars increased in weight only about 50 percent and essentially all of this weight was due to the secondary cementum and dentin. This is indicated by the relative fluoride contents of the dentin and of the enamel in the molars versus the incisors.

SUMMARY

When rats are given fluoride at the level of 100 p.p.m. in the drinking water under the conditions of this experiment, the calcium content of the bone is not increased with the increase in fluoride content. It was also shown that there is a tendency for the magnesium content of the tissues to increase with increase in fluoride content.

Additional evidence has been obtained to show that the material formed by the

in vitro reaction of fluoride ions with dentin and bone may be fluorapatite probably produced by ionic exchange and recrystallization, and shows reactions similar to that produced by the in vivo reaction with fluoride (solubility and hydroxyl exchange). However, when enamel it treated in vitro the reaction presumably takes place mostly on the outer surfaces because of the inability of fluoride ion to penetrate to the hidden area of the crystal. This is probably because of the extremely slow thermal aging of enamel as opposed to that of bone and dentin. Fluoride, however, is incorporated throughout the tissue in the in vivo reaction.

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DEPOSITION OF FLUORIDE, CALCIUM AND PHOSPHORUS IN EXPERIMENTAL LOW-PHOSPHORUS RICKETS

I. Zipkin, R. C. Likins, and F. J. McClure

It has been shown that 95 percent of the fluoride present in an animal carcass is found in the bones (Lawrenz et al., 1940) and thus it is of interest to observe the deposition of fluoride in these tissues in various types of bone dyscrasias. Perhaps the most widely studied disease affecting calcification is rickets. The deposition of fluoride in rachitic animals has been reported in two studies both of which pertained to elevated levels of fluoride (Schultz, 1936; Kempf and Nelson, 1941). No data were presented in one study (Shultz, 1936) and the deposition of fluoride was determined in the other (Kempf and Nelson, 1941) only during a short period of healing. It has been noted also that 300 p.p.m. of fluoride reduced the severity of developing rickets by increasing bone density (Morgareidge and Finn, 1940), and, in addition, the life span of rachitic rats appeared to be increased (Finn and Kramer, 1940).

The purpose of the present experiments, therefore, was to study the deposition of fluoride in rachitic rats receiving a low level of fluoride in the drinking water. In addition, data are presented on the fat, ash, calcium and phosphorus content of the femurs, mandibles, and teeth. The design of the study permits the separation of the effects of rickets *per se* and inanition.

EXPERIMENTAL

A diet adequate in calcium and vitamins and extremely limited in phosphorus (0.40% Ca and 0.017% P) was first proposed by Day and McCollum (Day and McCollum, 1939; Follis et al., 1940) and later modified (Coleman et al., 1950, 1953; Cramer et al., 1956) as a simplified approach to the production of experimental rickets. The diet as modified by Coleman et al. (1950) was employed in this study and contained 0.442% Ca, 0.018% P and 2.8 p.p.m. F. The control diet was supplemented with Na_2HPO_4 to provide adequate phosphorus and an adequate Ca/P ratio and on analysis was found to contain 0.437% Ca, 0.559% P and 3.0 p.p.m. F.

Ten litters of three female weanling, Sprague-Dawley rats each were divided into three equal groups as follows: group I received the rachitogenic diet and groups II and III received the rachitogenic diet supplemented with Na_2HPO_4. Group II was pair-fed to group I, and group III was fed ad libitum. The fluoride intake which was provided in the drinking water was approximately equalized for all groups. This was accomplished by periodic changes in the fluoride content of the water. Most of the time the water contained 20 p.p.m. of fluoride. The rats were individually housed in screen-bottom cages and the diet intakes were recorded. The rats were roentgenographed after three weeks and sacrificed at 6 weeks. The mandibles and femurs were dissected free of soft tissue, and molars and incisors removed. All samples were dried overnight at 105° C. ashed for 3 hours at 550° C. dissolved in 2 ml. of 1.0 N HCl per 100 mg. of ash and diluted to an appropriate volume. Suitable aliquots were removed and analyzed for fluoride (Willard and Winter, 1933, as modified by Armstrong, 1936 and McClure, 1939), calcium and phosphorus (A.O.A.C., 1955, p. 102 and 115). Representative samples of the dry bones and teeth were extracted for 8 hours with alcohol and for 4 hours with ether for the determination of fat.

RESULTS AND DISCUSSION

A. General. The mean weight gains, diet and fluoride intakes and dry weights of the femurs, mandibles, molars and incisors are presented in table 1. The three groups of rats had a mean fluoride intake of 12.3 to 13.2 mg. Although receiving equal quantities of diet, the pair-fed nonrachitic rats gained significantly more than their rachitic pair mates. The rats fed ad libitum and receiving the phosphorus-supplemented diet showed adequate weight gains of 2.3 gm./day and food utilization at the rate of 0.27 gm. weight per gram of diet.

It was evident from the roentgenographs that the rats receiving the low-phosphorus diet had developed rickets as previously described, whereas rats given the phorphorus-supplemented diet were normal. Characteristic of classical rickets, the femurs and mandibles of the rachitic rats weighed markedly less than those of their nonrachitic litter mates. Only small weight differences occurred in the teeth.

The mean concentration of fat of the femurs of five randomly selected rats from each group was: rachitic, $17.7 \pm 1.4\%$; pair-fed, $9.3 \pm 1.5\%$; and ad libitum-fed, $5.1 \pm 0.6\%$. Similarly the mandibles contained $8.8 \pm 0.4\%$, $3.8 \pm 0.4\%$ and $2.9 \pm 0.6\%$ respectively. The molars contained $1.7 \pm 0.2\%$, $1.6 \pm 0.4\%$, $1.9 \pm 0.2\%$, and the incisors $1.3 \pm 0.3\%$, $1.0 \pm 0.1\%$ and $1.3 \pm 0.1\%$ fat respectively. The femurs and mandibles of the rachitic rats thus contained markedly higher concentrations of fat than the same bones of the nonrachitic rats, whereas no change was found in the molars or incisors.

As shown in table 2, the ash concentration of the bones of the rachitic rats was markedly less than that of their nonrachitic pair-fed litter mates ($P < 0.001$) whereas smaller reductions were seen in the ash concentration of the molars and incisors ($P < 0.05$).

B. Deposition of fluoride in the bones and teeth. Although the bone ash of the rachitic rats showed a 2½-fold increase in fluoride concentration when compared with their nonrachitic pair-fed mates (table 2), only about half as much total fluoride was found. No differences in deposition of fluoride were apparent in the molars and incisors of the rachitic and their nonrachitic pair-fed litter mates.

Differences between the rachitic and the nonrachitic pair-fed litter mates represent the effect of rickets since the diet intakes were equalized. On the other hand, variations between the pair-fed and ad libitum-fed nonrachitic rats are attributed to inanition. No differences were found in the concentration of ash and fluoride in either the mandibles or the molars of the two groups of nonrachitic rats and only small differences were seen in the femurs and incisors (table 2). Differences in ash and deposition of fluoride in the present study are therefore due to the rachitogenic diet rather than to inanition.

TABLE 1.—*Weight gains, diet and fluoride intakes, and weights of skeletal and dental tissues of rats receiving a low phosphorus rachitogenic diet supplemented with* Na_2HPO_4

	RATS RECEIVING RACHITOGENIC DIET [1]	RATS RECEIVING RACHITOGENIC DIET + Na_2HPO_4, PAIR-FED	RATS RECEIVING RACHITOGENIC DIET + Na_2HPO_4, AD-LIBITUM-FED
Weight gain, (gm/day)[2]	0.5 ± 0.02 [3]	0.7 ± 0.04	2.3 ± 0.1
Diet intake, (gm/day)	4.6 ± 0.1	4.4 ± 0.1	8.3 ± 0.2
F intake, (mg)	12.8 ± 0.5	12.3 ± 0.4	13.2 ± 0.3
Oven-dry weights of skeletal and dental tissues			
	gm	*gm*	*gm*
Femurs	0.239 ± 0.012	0.430 ± 0.008	0.589 ± 0.015
Mandibles	0.091 ± 0.004	0.212 ± 0.005	0.250 ± 0.009
Molar teeth	0.104 ± 0.001	0.116 ± 0.002	0.124 ± 0.005
Incisor teeth	0.149 ± 0.004	0.177 ± 0.002	0.191 ± 0.003

[1] Initial mean weight of each of the three groups of 10 rats was 37 gm.

[2] Duration of study was 42 days.

[3] Standard errors calculated according to Mantel ('51).

The markedly higher concentration of fluoride but lower total fluoride in rachitic bones as compared to bones of nonrachitic rats is explained largely by the smaller amount of bone ash present in rachitic bones. This situation would furnish fewer exchangeable sites for fluoride per unit volume of rachitic bone. It is likewise possible that differences in composition of the bone mineral itself, as evident in the Ca/P ratio (table 3) could play some part in skeletal fluoride deposition. Other mechanisms such as fluoride incorporation in forming bone and recrystallization (Neuman and Neuman, 1950) may also contribute to differences in fluoride retained by calcified tissues.

No data on the deposition of fluoride in severe phosphorus deficiency appear to be available. The deposition of fluoride in rats receiving diets containing less drastic changes in the Ca/P ratio and also containing low levels of fluoride has been reported by Lawrenz and Mitchell (1941). They found that increasing the phosphorus content from 0.14 to 0.71 percent in a diet containing 0.71 percent of Ca plus adequate amounts of vitamins A and D increased the weight of the bones but decreased the concentration of fluoride, so that the total fluoride content was unchanged. The rats received 13 and 32 p.p.m. of fluoride in the diet. The phosphorus deficiency of their diet evidently was not sufficient to produce the marked changes in fluoride deposition observed in the present study where the diet contained only 0.018 percent of P. The effects of simple inanition in the present study parallel those of Lawrenz and Mitchell (1941) on the low-phosphorus diet in that the total F deposited in the bones and teeth was similar in the rats on either the pair-fed or ad libitum regimen.

C. Calcium and phosphorus in the bones and teeth. The ash of the femurs and mandibles of the rachitic rats was lower in calcium and higher in phosphorus, so that a markedly lower Ca/P weight ratio was found than in the same bones of the nonrachitic pair-fed rats. No changes were observed in either the bones of the two nonrachitic groups or in the molar and incisor teeth of all three groups.

Although a large body of data is available on the ash, calcium and phosphorus content of the bones and teeth of rachitic rats and requires no additional review (Karshan, 1933, Karshan and Rosebury, 1933, Shohl et al., 1933, Sobel and Hanok, 1948), it cannot be compared unequivocally with the present data because of the imposed ingestion of fluoride in the present study. The marked resistance to change in the calcium and phosphorus concentration of the molars and incisors in this study has been observed with other stress diets (Karshan, 1933; Karshan and Rosebury, 1933; Wynn et al., 1957; McClure, 1958). Fluoride deposition in the teeth also was not affected in the present study by the rachitic state of the animal.

Rachitic rats as compared to their nonrachitic, pair-fed mates showed the greatest changes in the femurs, followed by the mandibles. Minor or no differences were observed in the various constituents between the nonrachitic pair-fed and ad libitum fed rats. Changes in the rachitic rat were due, therefore, to the extreme phosphorus deficiency of the diet, rather than to simple inanition.

SUMMARY

1. Rats were rendered rachitic on a diet adequate in vitamins A and D and in calcium but markedly deficient in phos-

TABLE 2.—*Deposition of fluoride in the ash of the bones and teeth*

	RACHITIC (R)	PAIR-FED (PF)	AD. LIB. FED (ALF)	P VALUES [2]	
				R vs PF	PF vs ALF
			Femurs		
% Ash, oven dry	18.28 ± 0.75 [1]	48.20 ± 0.49	51.43 ± 0.82	< 0.001	< 0.001
% F, ash	0.435 ± 0.015	0.179 ± 0.007	0.150 ± 0.006	< 0.001	> 0.05
Total F, mg	0.187 ± 0.009	0.370 ± 0.019	0.442 ± 0.029	< 0.001	< 0.05
			Mandibles		
% Ash, oven dry	31.77 ± 1.31	57.84 ± 0.55	57.11 ± 1.07	< 0.001	> 0.05
% F, ash	0.410 ± 0.024	0.163 ± 0.008	0.131 ± 0.004	< 0.001	> 0.05
Total F, mg	0.116 ± 0.005	0.199 ± 0.011	0.181 ± 0.010	< 0.001	> 0.05
			Molars		
% Ash, oven dry	74.32 ± 0.65	76.28 ± 0.38	76.17 ± 0.37	< 0.05	> 0.05
% F, ash	0.069 ± 0.007	0.060 ± 0.003	0.055 ± 0.003	> 0.05	> 0.05
Total F, mg	0.055 ± 0.004	0.053 ± 0.004	0.052 ± 0.004	> 0.05	> 0.05
			Incisors		
% Ash, oven dry	73.53 ± 0.58	75.45 ± 0.69	76.42 ± 0.31	< 0.05	> 0.05
% F, ash	0.103 ± 0.007	0.090 ± 0.005	0.067 ± 0.003	> 0.05	< 0.01
Total F, mg	0.117 ± 0.007	0.121 ± 0.007	0.098 ± 0.005	> 0.05	< 0.05

[1] Standard error.

[2] Calculated by analysis of variance for 10 litter-mate trios.

TABLE 3.—*Concentration of calcium and phosphorus in the ash of the bones and teeth*

	RACHITIC (R)	PAIR-FED (PF)	AD. LIB. FED (ALF)	P VALUES [2]	
				R vs PF	PF vs ALF
			Femurs		
Ca	33.11 ± 0.47 [1]	36.79 ± 0.13	36.91 ± 0.35	< 0.001	> 0.05
P	20.37 ± 0.29	18.57 ± 0.24	18.22 ± 0.18	< 0.001	> 0.05
Ca/P	1.63 ± 0.02	1.98 ± 0.02	2.03 ± 0.02	< 0.001	> 0.05
			Mandibles		
Ca	35.05 ± 0.73	37.47 ± 0.46	37.10 ± 0.26	< 0.01	> 0.05
P	19.34 ± 0.17	18.80 ± 0.32	18.94 ± 0.16	> 0.05	> 0.05
Ca/P	1.82 ± 0.05	2.00 ± 0.02	1.96 ± 0.02	< 0.001	> 0.05
			Molars		
Ca	38.14 ± 0.21	37.70 ± 0.27	37.94 ± 0.28	> 0.05	> 0.05
P	19.37 ± 0.20	19.53 ± 0.27	19.50 ± 0.36	> 0.05	> 0.05
Ca/P	1.97 ± 0.03	1.93 ± 0.03	1.95 ± 0.03	> 0.05	> 0.05
			Incisors		
Ca	35.85 ± 0.15	35.65 ± 0.11	35.28 ± 0.25	> 0.05	> 0.05
P	20.12 ± 0.28	21.01 ± 0.29	21.80 ± 0.63	> 0.05	> 0.05
Ca/P	1.79 ± 0.03	1.70 ± 0.03	1.63 ± 0.03	< 0.05	> 0.05

[1] Standard error.

[2] Calculated by analysis of variance for 10 litter-mate trios.

phorus. Litter-mate rats received the same diet supplemented with Na_2HPO_4. An equalized intake of fluoride was provided by the drinking water. Ash, fat, fluoride, calcium and phosphorus were determined in femurs, mandibles, molar and incisor teeth.

2. The bones of the rachitic rats showed a marked reduction in ash, whereas the teeth were essentially normal. The bones of the rachitic rats also contained less calcium but more phosphorus, resulting in a highly significant reduction in the Ca/P ratio. There was no change in the calcium or phosphorus concentration of the teeth of rachitic rats, nor in the Ca, P or Ca/P ratio in the bones and teeth of the nonrachitic pair-fed and ad libitum-fed rats.

3. The femur and mandible ash of the rachitic rats was increased 2½ times in fluoride content as compared with the bone ash of nonrachitic litter mates. However, markedly less total fluoride was present in the rachitic bones. Fluoride in the teeth was not altered by the rachitic state of the animals.

4. The data permitted the conclusion that the observed changes in the rachitic rats were due to the extremely low phosphorus content of the diet rather than to inanition.

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DEPOSITION AND RETENTION OF FLUORIDE AND RADIOCALCIUM IN THE GROWING RAT

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ABSTRACT

Radiocalcium and fluoride were administered daily for 30 days to young rats. At 30 and 60 days, the tibia, femur, mandible, teeth and remaining skeleton were analyzed for Ca^{45} and F. The tibia was divided into several segments including the proximal epiphysis, the original diaphyseal segment, and those portions of the diaphysis added from 0 to 30 days and 30 to 60 days. Fluoride had no effect on the Ca^{45} or ash of bone but reduced Ca^{45} uptake by molar enamel. Ratios of F to Ca^{45} were dissimilar in the various tibial segments. Between 30 and 60 days essentially no Ca^{45} or F was lost from the skeleton, tibia or tibial segments excepting the 0–30-day proximal diaphyseal increment. Seventy percent of the Ca^{45} and 40 percent of the F lost from this segment was found in the adjacent segment formed during the second 30-day period. These results suggest that much of the Ca^{45} and F released during remodeling of the ends of the growing bone did not enter the general circulation, but was deposited in the immediate vicinity.

Although considerable attention has been paid to the skeletal and dental changes in chronic fluorosis, the role of fluoride in calcification remains obscure. Recently, some progress has been made toward elucidating this problem through the use of radioactive calcium. Thus, Cicardo et al. (*1*) have shown that the daily administration of fluoride to rats resulted in an increased uptake of radiocalcium by the long bones, while Comar et al. (*2*) found an increased resorption of calcium^{45} from the femora of fluoride intoxicated pigs. In the present study radioactive calcium was employed to investigate the effect of fluoride on calcium metabolism in various parts of the tibia, in whole bones, and in the dentin and enamel of the molar and incisor teeth.

EXPERIMENTAL METHODS

Littermates from 15 litters of weanling female Sprague-Dawley rats were assigned to four groups. Those belonging to two groups were given 0.33 mg. of fluoride (NaF) in 1.0 ml. of distilled H_2O daily by stomach tube for 30 days; the others were intubated with distilled H_2O only. All received 0.7 μc of $Ca^{45}Cl_2$ (in 1.0 ml. distilled H_2O) intraperitoneally approximately 30 minutes after each intubation. On the 30th day, one fluoride-treated (*F-I*) and one control (*C-I*) group were killed. The remaining groups, *F-II* and *C-II*, were continued for an additional 30 days, during which time neither radiocalcium nor fluoride was administered. The rats, individually housed in screen-bottom cages, were given a low-fluoride ration (*3*) and distilled water ad libitum. The initial and terminal weight and the food consumption of each rat were recorded.

To facilitate the study of bone formed during the experimental periods, the tibiae were divided into several segments by a previously described technique (*4*). At the beginning of the experiment a small, stainless-steel pin was surgically inserted into the middle third of the anteromedial aspect of the left tibia of each animal as a fixed point of reference. The animals were then radiographed and the distance from the proximal end of the pin to the proximal and distal epiphyseal lines measured on the roentgenograms. After 30-day and 60-day intervals, the pin-to-epiphyseal line distances were determined in the same manner. The gain in length of the diaphysis at each end for each period was obtained by difference (fig. 1). At autopsy, the tibiae were excised and the proximal epiphyses were removed by sectioning through the epiphyseal plate. From the proximal end of the diaphysis the following segments were marked off on both tibiae: the 30–60-day proximal diaphyseal growth increment (*F-II* and *C-II*); the 0–30-day proximal diaphyseal growth increment (all groups); the "original" diaphyseal segment (all groups). Since there were no differences in length between the 2 tibiae in each animal, the measurements from the left tibia were used to mark off the segments of the other tibia. The bones were then sectioned transversely at the marks to give the segments shown diagrammatically in figure 2. For analyses, the distal diaphyseal increments were combined with the distal epiphysis.

Immediately prior to killing, the lymphocytes and polymorphonuclear cells were counted (*5*) in the 60-day control and fluoride-treated animals to determine if there had been any radiation damage.

At autopsy, the incisor and molar teeth were extracted and the femurs and mandibles excised. The remaining skeleton was cleaned of adherent soft tissue by larvae of *Dermestes lardarius* (*6*). With the exception of the femurs which were pooled, the various bone samples, including the several tibial segments obtained from each rat, were analyzed separately for radiocalcium as follows:

The samples were extracted with alcohol (8 hr.) and ether (4 hr.), dried overnight at 105° C, and incinerated at 550° C for 3 hours. To facilitate sampling, the ash of the remaining skeleton was ground to pass through a 60-mesh sieve. The samples were dissolved at room temperature in 1 N HCl (0.02 ml./mg. ash). These solutions were then diluted and aliquots taken for counting which would give a sample thickness when dried of 0.1 mg. ash/cm^2. Radioactivity was measured with an end window Geiger-Müller tube. A sufficient number of counts was obtained to insure a counting error of less than 2 percent.

The solutions of bone ash were analyzed for fluoride by standard procedures (*7, 8*). When required, the ash of corresponding right and left segments from the same animal were pooled to provide sufficient material for analysis.

The incisor and molar teeth from each rat were pooled separately, ground to pass through a 60-mesh screen, and the enamel separated from the dentin by flotation (*9*). The samples were dried in air at 105° C, and the ash residues analyzed for radiocalcium and fluoride in the same manner as for bone.

The differences between means were compared for significant levels according to Fisher's *t* test for paired values (*10*).

RESULTS

Except for three control and two fluoride-treated rats which died during the first 4 days, all were in good health throughout the experiment. The only gross observation of note was the appearance of incisor fluorosis in the treated groups. There were no significant differences in the mean body weight and food consumption between the various groups at the end of 30 and 60 days. The white cell concentration in the peripheral blood was within normal limits (*11*), indicating that radiation had no effect on leucocytopoiesis.

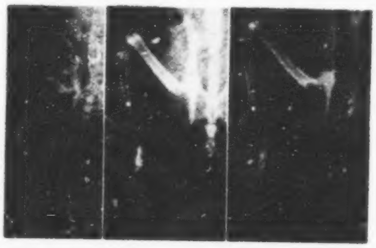

Figure 1.—*Position of pin in tibia at start and at end of 30 and 60 days on experiment.*

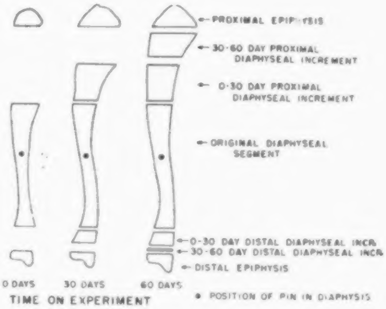
PROXIMAL EPIPHYSIS
30-60 DAY PROXIMAL DIAPHYSEAL INCREMENT
0-30 DAY PROXIMAL DIAPHYSEAL INCREMENT
ORIGINAL DIAPHYSEAL SEGMENT
0-30 DAY DISTAL DIAPHYSEAL INCR.
30-60 DAY DISTAL DIAPHYSEAL INCR.
DISTAL EPIPHYSIS
0 DAYS
30 DAYS
60 DAYS
TIME ON EXPERIMENT
• POSITION OF PIN IN DIAPHYSIS

Figure 2.—*Segments of tibia.*

Tibia Segments

The pin marker inserted in the left tibia had no effect on the length or ash content of the bone. The mean length (cm.) of the left and right diaphysis at zero, 30, and 60 days for all groups of rats was 1.60, 1.61; 2.54, 2.54; and 3.01, 3.02, respectively. The mean ash content (mg.) of the left and right diaphysis at the end of 30 and 60 days was 74.0, 74.7; and 132.7, 133.7, respectively.

Growth of diaphysis.—Growth at the proximal end accounted for nearly three-quarters of the increase in diaphyseal length during the initial 30 days (0.69 cm. and 0.24 cm. for the proximal and distal ends, respectively) and more than 90 percent during the succeeding 30-day period (0.41 cm. and 0.04 cm., respectively). Growth at the distal end had ceased by the 30th day.

Ash content.—There was no effect of fluoride administration on the ash content of all tibial segments studied except that the total ash content of the proximal epiphysis of rats of *period II* was slightly higher in the fluoride-treated groups (table 1).

Radiocalcium content.—At the end of

the first 30 days, the radiocalcium concentration of the original diaphyseal segment in the control and fluoride-treated group was significantly less than that of the other segments, i.e., 0.035 vs. 0.041–0.042 percent of the administered dose per milligram of ash (table 1). With the exception of the proximal epiphysis of rats of *period II*, fluoride treatment had no effect on either the percentage or total radiocalcium of the various segments.

Fluoride content.—There was a different concentration of fluoride in each of the tibial segments at the end of 30 days. The ratios of fluoride to radiocalcium for the various segments were also dissimilar (table 1). For example, this ratio was 9.60 in the proximal epiphysis and 7.67 in the "original" diaphyseal segment at the end of *period I*.

The concentration of fluoride and radiocalcium in the various segments decreased during the second 30-day period (table 1). With the exception of the 0–30 day proximal diaphyseal increment, these decreases are the result of dilution with new bone mineral. The decreases in the 0–30 day proximal diaphyseal increment are due in part to the loss of fluoride and radiocalcium from this segment.

Local retention of fluoride and radiocalcium.—The proximal epiphysis, original diaphyseal segment and the distal fragment neither gained nor lost fluoride and radiocalcium during the second 30-day period (table 1). The 0–30-day proximal diaphyseal increment, however, lost approximately half of the fluoride and radiocalcium that was present at the end of the first 30-day period. About 41 percent of the fluoride and 69 percent of the radiocalcium lost by the 0–30-day proximal diaphyseal increment could be accounted for by the fluoride and radiocalcium deposited in the 30–60-day proximal diaphyseal increment. The concentration of radiocalcium in the 30–60-day proximal diaphyseal increment was approximately one-half of that of the other segments whereas the concentration of fluoride was one-fourth to one-half. It is of interest that the percentage of fluoride in this segment of the treated group is approximately 10 times that of the residual skeleton of the control groups (table 2).

TIBIA, FEMUR, MANDIBLE AND SKELETON

Ash content.—The data for percentage and total ash indicate that fluoride treatment did not significantly affect the mineral content of the various bone specimens (table 2).

Fluoride content.—There was little difference between the fluoride concentrations of the different bones at the end of either 30 days or 60 days (table 2). Upon statistical analysis, however, the mandible in each instance was found to have a lower ($P < 0.05$) concentration of fluoride than the other bone samples.

Radiocalcium content.—At the end of each experimental period the percentage of radiocalcium in the ash was quite similar in the different bone samples (table 2). Fluoride treatment had no effect on either the concentration or total radiocalcium of the various bones.

Retention of fluoride and radiocalcium.—At the end of 30 days, the total skeleton of the control and fluoride-treated groups contained 83.3 percent and 82.1 percent, respectively, of the total radiocalcium administered, whereas only about 60 percent of the fluoride supplement was retained (table 2). During the next 30 days there was essentially no change in total skeletal fluoride or radiocalcium in either the control or treated group.

TABLE 1.—*Content of ash, fluoride and calcium*^{45} *in different segments of tibia*

Sample	Period	Group	Ash, mg	Ash, %*	F, % of Ash	Total F in Ash, µg	Ca^{45}, % Dose/mg Ash	Total Ca^{45}, % Dose	F, % of Ash / Ca^{45}, % Dose/mg Ash
Proximal epiphysis	*I*	*C*	6.9±0.3†	36.91±0.70			0.0409±0.0016	0.281±0.015	
		F	6.9±0.5 (12)‡	37.66±0.60 (12)	0.398±0.013 (12)	27.1±1.6 (12)	0.0420±0.0013 (12)	0.283±0.013 (12)	9.60±0.16 (12)
	II	*C*	12.9±0.8	49.63±0.68			0.0235±0.0009	0.295±0.013	
		F	14.5±0.5§ (12)	49.34±0.54 (11)	0.224±0.006 (10)	31.1±0.8 (10)	0.0215±0.0005§ (12)	0.308±0.009 (12)	10.13±0.19 (10)
0–30-Day proximal diaphyseal increment	*I*	*C*	20.5±1.0	49.02±0.88			0.0417±0.0016	0.842±0.032	
		F	20.7±0.7 (12)	50.49±0.72 (12)	0.306±0.009 (12)	62.6±1.5 (12)	0.0422±0.0012 (12)	0.862±0.015 (12)	7.36±0.11 (12)
	II	*C*	21.9±2.3	66.59±0.70			0.0212±0.0008	0.451±0.016	
		F	23.7±1.5 (12)	67.69±0.45 (12)	0.120±0.003 (11)	28.8±0.4 (10)	0.0195±0.0004 (12)	0.457±0.017 (12)	6.10±0.10 (11)
30–60-Day proximal diaphyseal increment	*II*	*C*	22.9±1.0	57.72±0.93			0.0126±0.0007	0.281±0.006	
		F	24.8±0.7 (12)	57.75±0.89 (12)	0.057±0.002 (9)	13.8±0.6 (9)	0.0113±0.0003 (11)	0.278±0.010 (11)	4.94±0.14 (8)
Original diaphyseal segment	*I*	*C*	35.5±1.8	63.72±0.38			0.0347±0.0011	1.214±0.026	
		F	34.3±1.1 (12)	64.99±0.31 (12)	0.279±0.008 (12)	94.7±2.3 (12)	0.0365±0.0011 (12)	1.234±0.015 (12)	7.67±0.14 (12)
	II	*C*	49.3±2.0	69.78±0.51			0.0275±0.0011	1.337±0.036	
		F	51.9±2.5 (11)	70.26±0.20 (11)	0.178±0.006 (9)	92.9±2.5 (9)	0.0261±0.0005 (10)	1.375±0.059 (10)	6.89±0.16 (9)
Distal epiphysis and distal diaphyseal increment	*I*	*C*	12.3±0.4	48.73±0.37			0.0418±0.0016	0.508±0.013	
		F	11.8±0.4 (12)	50.32±0.42 (12)	0.354±0.011 (12)	41.1±0.9 (12)	0.0422±0.0013 (12)	0.490±0.010 (12)	8.39±0.19 (12)
	II	*C*	21.4±1.3	56.93±0.83			0.0260±0.0009	0.554±0.022	
		F	22.4±0.6 (12)	57.53±0.58 (12)	0.195±0.004 (10)	45.0±2.7 (10)	0.0245±0.0006 (12)	0.547±0.014 (12)	7.81±0.17 (10)

* Based on weight of alcohol and ether extracted sample. † Standard error. ‡ Number of values comprising mean. For fluoride analyses, right and left segments from each animal pooled in following cases: *period I*, proximal epiphysis, distal epiphysis and distal diaphyseal increment(s): *period II*, proximal epiphysis, proximal diaphyseal increment (0–30-day), proximal diaphyseal increment (30–60-day), distal epiphysis and distal diaphyseal increment(s). § Probability <0.05.

INCISOR AND MOLAR TEETH

Ash content.—Fluoride treatment had no effect on the percentage of ash of the dentin and enamel of the incisor and molar teeth (table 3). While the enamel of *period II* rats, both incisor and molar, appeared to have a slightly lower percent ash than the enamel of *period I* animals, consideration must be given to the following: the enamel samples of *period I* rats were freed of the bromoform-acetone mixture and acetone rinses by decantation. This procedure resulted in small losses of enamel and it was decided to aspirate these solutions from the enamel of *period II* rats. It is possible that the portions lost through decantation were rich in the lower-density junction particles, thus accounting for elevated ash values in the remaining samples.

Fluoride content.—Within experimental error, the molar and incisor dentin of the 30-day treated rats contained the same percentage of fluoride (table 3). At the end of the second 30-day period, however, the fluoride content of the incisor dentin was considerably less than the molar dentin, i.e. 0.044 percent vs. 0.132 percent.

Radiocalcium content.—Fluoride administration did not affect the concentration of radiocalcium in the molar and incisor dentin (table 3). It will be noted, however, that the percentage of radiocalcium in the molar enamel was significantly less ($P < 0.01$) in the fluoride-treated rats than in the controls, at the end of both 30 days and 60 days. During the second 30-day period the concentration of radiocalcium, like that of fluoride, decreased more in the incisor dentin than in the molar dentin. At the end of 60 days the concentration of radiocalcium in the incisor enamel was half that present at 30 days. There was no apparent change with time in the concentration of radiocalcium in the molar enamel.

DISCUSSION

During the second 30-day period, when neither Ca^{45} nor F was given, there was no significant loss of these ions from any of the skeletal structures studied except the 0–30-day proximal diaphyseal segment of the tibia. One of the most interesting findings in this study was that the equivalent of 70 percent of the radiocalcium and 40 percent of the fluoride lost from this segment was found in the adjacent segment newly formed during this period, namely the 30–60-day proximal diaphyseal increment. The original diaphyseal segment and proximal epiphysis showed little change in Ca^{45} and F during this period. These findings would suggest that most of the calcium and fluoride released during the remodeling of the ends of growing bone is deposited in the immediate vicinity without entering the general circulation.

Two observations in support of this hypothesis are the following: the total loss of Ca^{45} and F from the whole skeleton during the 30–60-day period was very small. This might be expected if the turnover of these ions was small. Assuming that all released Ca^{45} enters the general circulation and that all ash formed throughout the skeleton during the 30–60-day period has the same radioactivity as that found in the 30–60-day proximal diaphyseal segment, 0.011–0.012 percent of dose per milligram of ash, then the proximal epiphysis, which doubled in ash content, should have had a gain in total Ca^{45} equivalent to 0.08 percent of the dose. The observed gain, however, was only one-sixth of that expected. The fact that there was essentially no change in the radiocalcium

TABLE 2.—*Content of ash, fluoride and calcium*^{45} *in tibia, femur, mandible, residual and total skeleton*

Sample	Period	Group	Ash, mg	Ash, %[a]	F, % of Ash	Total F in Ash, mg	Ca^{45}, % Dose/mg Ash	Total Ca^{45}, % Dose	F, % of Ash / Ca^{45}, % Dose/mg Ash
Tibia[b]	*I*	*C*	75.1±3.1[c]	52.97±0.58			0.0386±0.0017	2.844±0.05	
		F	73.6±2.2 (12)[d]	54.47±0.37 (12)	0.311±0.009 (12)	0.227±0.003 (12)	0.0395±0.0011 (12)	2.869±0.02 (12)	7.90±0.093 (12)
	II	*C*	129.1±5.3	61.96±0.71			0.0232±0.0008	2.903±0.05	
		F	137.4±4.8 (12)	62.70±0.28 (12)	0.154±0.004 (7)	0.212±0.002 (7)	0.0215±0.0003 (12)	2.947±0.08 (12)	7.21±0.17 (7)
Femur	*I*	*C*	90.3±4.1	51.62±0.62			0.0416±0.0016	3.695±0.07	
		F	86.9±2.9 (12)	51.43±0.67 (12)	0.314±0.007 (12)	0.270±0.004 (12)	0.0415±0.0011 (12)	3.559±0.04 (12)	7.60±0.10 (12)
	II	*C*	161.1±6.8	59.93±0.43			0.0233±0.0007	3.683±0.07	
		F	170.8±5.4 (13)	60.82±0.44 (13)	0.147±0.003 (13)	0.250±0.004 (13)	0.0219±0.0005[f] (13)	3.719±0.04 (13)	6.73±0.082 (13)
Mandible	*I*	*C*	79.6±3.2	61.30±0.58			0.0382±0.0014	3.012±0.08	
		F	76.1±2.4 (12)	60.44±0.41 (12)	0.298±0.007 (12)	0.225±0.005 (12)	0.0390±0.0010 (12)	2.947±0.08 (12)	7.52±0.18 (12)
	II	*C*	148.2±4.1	68.06±0.30			0.0215±0.0008	3.148±0.07	
		F	159.6±4.3[f] (13)	68.83±0.30 (13)	0.132±0.003 (13)	0.209±0.005 (13)	0.0203±0.0005 (13)	3.225±0.06 (13)	6.48±0.10 (13)
Residual skeleton	*I*	*C*	1697.0±80.2	54.05±0.36	0.006[g]		0.0370±0.0019	62.254±1.20	
		F	1508.4±97.8 (6)	54.03±1.19 (6)	0.318±0.009 (10)	4.822±0.093 (8)	0.0410±0.0022 (6)	60.946±0.57 (6)	8.15±0.13 (10)
	II	*C*	2860.5±151.8	60.39±0.33	0.005[g]		0.0210±0.0010	58.955±1.05	
		F	3112.1±100.4 (10)	61.13±0.21 (10)	0.150±0.004 (10)	4.658±0.093 (10)	0.0193±0.0005 (10)	59.753±0.89 (10)	7.79±0.076 (10)
Total skeleton[h]	*I*	*C*	2278.2±99.0	55.28±0.33			0.0369±0.0017	83.250±1.41	
		F	2040.7±122.4 (6)	55.24±0.93 (6)	0.308±0.011 (8)	6.234±0.095 (8)	0.0407±0.0021 (6)	82.136±0.47 (6)	7.68±0.070 (8)
	II	*C*	3783.3±188.9	61.49±0.30			0.0213±0.0010	79.166±1.21	
		F	4101.7±132.8 (10)	62.24±0.20[f] (10)	0.148±0.003 (8)	5.978±0.075 (8)	0.0196±0.0005 (10)	80.072±1.02 (10)	7.39±0.10 (8)

[a] Based on weight of alcohol and ether extracted sample. [b] Reconstituted from segments. [c] Standard error. [d] Number of values comprising mean. [f] Probability <0.05. [g] Analysis based on pooled samples. [h] Included teeth.

content of the proximal epiphyses would also indicate that calcium released from the ends of the diaphysis did not enter the general circulation. The small gain in the total Ca^{45} of the original diaphyseal segment during the 30–60-day period was not significantly different from that expected on the basis of its gain in ash. This is also compatible with the idea of local circulation of the released ions during the active growth period of bone. More specific experiments are needed to establish this point.

Differences in the fluoride: calcium^{45} ratios of the tibial segments at the end of 30 days may be due to the disproportionate uptake of these ions, their unequal loss, or both. While additional definitive data are required for the evaluation of these possibilities, it seems unlikely that the deposition of new bone mineral per se is responsible for the different fluoride: calcium^{45} ratios. This is evident from a comparison of the relative concentrations of these ions in the 0–3-day proximal diaphyseal increment, and in the original diaphyseal segment. Thus if new crystal formation favored the deposition of fluoride over calcium^{45}, an expected finding would have been a higher fluoride: calcium^{45} ratio in the more rapidly calcifying segment, that is, in the proximal diaphyseal increment. This was not the case. Furthermore, since newly deposited bone mineral recrystallizes faster than older bone (*12*), it is equally improbable that recrystallization is a factor in the differences found.

As previously mentioned, differences in the 30-day fluoride: calcium^{45} ratios could result from the unequal loss of these ions. Supporting this is the finding that while the concentration of radiocalcium was approximately the same in all of the segments, the concentration of fluoride was highest in the "ends", that is, in the proximal epiphysis and in the distal fragment. Previous experiments have shown that the initial incorporation of radiocalcium (*13*), as well as that of fluoride (*14, 15*), is greater in the ends than in the shaft. Accordingly, the lack of difference between ends and shaft as regards their radiocalcium content could reflect a preferential loss of calcium^{45} from the ends during the early stages of isotope incorporation. By the same reasoning, the greater disparity which exists between the ends and shaft for fluoride may be due to a relative inability of the fluoride ion to participate in exchange reactions, once deposited. Of interest in this connection are the results of *in vitro* studies which indicate that although the exchange-desorption reaction for calcium is freely reversible (*16*), fluoride can only be desorbed with difficulty (*17*).

It is clear that the fluoride supplement had no effect on the deposition of radiocalcium. The fact that the losses of radiocalcium were the same in both groups of rats also suggests that the increased fluoride content of the skeleton did not affect bone resorption or remodeling. These findings are of interest since the skeletal concentration of fluoride in the treated animals is at least as high as that found in individuals using a fluoridated drinking water (*18*).

The finding that fluoride administration resulted in a decreased uptake of radiocalcium by the molar enamel is noteworthy in view of the role of fluoride as a dental caries inhibitor. Presumably this effect is mediated through some alteration in the capacity of the enamel to exchange posteruptively since fluoride did not affect the deposition of labelled calcium in the still-calcifying enamel of the incisor.

While the concentration of fluoride at the end of 30 days was the same in the molar and incisor dentin, the latter contained nearly twice as much radiocalcium as its slower growing counterpart. This suggests that the rate of dentin formation may be of more importance in the fixation of radiocalcium than of fluoride. Thus, while the deposition of calcium^{45} may be accomplished primarily through its incorporation in forming crystals, other processes such as diffusion and exchange may play a relatively more significant role in the fixation of fluoride.

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TABLE 3.—*Content of ash, fluoride and calcium^{45} in enamel and dentin*

Sample	Period	Group	Ash, %[a]	F, % of Ash	Ca^{45}, % Dose/mg Ash	F, % of Ash / Ca^{45}, % Dose/mg Ash
Molar enamel	*I*	*C*	94.94±0.17[b]		0.0109±0.0005	
		F	94.78±0.23 (11)[d]		0.0090±0.0004[c] (10)	
	II	*C*	93.29±0.18		0.0115±0.0006	
		F	92.87±0.17 (13)		0.0089±0.0002[c] (13)	
Molar dentin	*I*	*C*	77.08±0.14	0.009[e]	0.0221±0.0007	
		F	77.01±0.24 (10)	0.152±0.003 (12)	0.0233±0.0005 (12)	6.61±0.082 (12)
	II	*C*	77.02±0.23	0.009[f]	0.0206±0.0007	
		F	77.23±0.18 (13)	0.132±0.005 (13)	0.0202±0.0006 (13)	6.52±0.16 (13)
Incisor enamel	*I*	*C*	94.68±0.37		0.0417±0.0013	
		F	94.58±0.35 (12)		0.0422±0.0023 (11)	
	II	*C*	93.35±0.19		0.0220±0.0009	
		F	93.42±0.14 (13)		0.0197±0.0010 (13)	
Incisor dentin	*I*	*C*	76.69±0.13	0.009[e]	0.0395±0.0017	
		F	77.18±0.16 (12)	0.150±0.004 (12)	0.0417±0.0012 (12)	3.61±0.062 (12)
	II	*C*	77.82±0.32	0.004[e]	0.0169±0.0008	
		F	77.86±0.30 (13)	0.044±0.002 (13)	0.0163±0.0007 (13)	2.73±0.057 (13)

[a] Based on weight of alcohol and ether extracted samples. [b] Standard error. [c] Probability <0.01. [d] Number of values comprising mean. [e] 10 pooled samples. [f] 12 pooled samples.

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QUANTITATIVE ANALYSIS AND CHEMICAL REACTIONS OF FLUORIDE

Analysis of Fluoride

Prior to 1933 there was no reliable procedure for the accurate quantitative determination of the microquantities of fluoride which occur in water, foods, and biologic materials. In that year H. H. Willard and O. B. Winter published a satisfactory new procedure, subsequently modified by W. M. Haskins, C. A. Ferris, W. D. Armstrong, R. J. Rowley, H. V. Churchill and others. For the estimation of fluoride in drinking water, a procedure first successfully modified by Elias Elvove, was of sufficient sensitivity to determine accurately as little as 0.1 p.p.m. of fluoride in water. Subsequent research and modification have improved the accuracy of this analytical technique.

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ESTIMATION OF FLUORIDES IN WATERS

By Elias Elvove, (C. G. Remsburg assisted in the determinations other than fluoride)

The association of the human dental lesion known as "mottled enamel" with fluoride in the drinking water (*2, 5, 6*) makes it desirable to have a convenient method for detecting and estimating fluorides in such waters. A colorimetric method would probably be most convenient and enable one to deal with comparatively very small quantities of fluoride.

It appeared that the zirconium-alizarin reagent of DeBoer (*4*) might be utilized in this connection. Willard and Winter (*8*), however, state that they attempted to use the colorimetric method based upon a zirconium-alizarin mixture but that their results did not show any definite relation between the fluorine content and the fading of the color. On the other hand, Casares and Casares (*1*) utilized this reagent for determining fluorides in mineral waters, and Thompson and Taylor (*7*) applied it to the determination of fluorides in sea water.

Casares and Casares used a series of standards ranging from 1 to 3.5 mg. of NaF in 100 cc., i.e. their lowest standard corresponded to about 0.45 mg. of fluorine in 100 cc. or 4.5 parts per million. Thompson and Taylor used a series of standards ranging from 0.08 to 0.16 mg. of flourine per 100 cc., which would indicate that by their improved procedure as little as 0.08 mg. of fluorine per 100 cc., or 0.8 parts per million, could be detected.

Thompson and Taylor acidified their waters by adding 2 cc. of 6 N HCl to 100 cc. of the sample, which is about the same hydrochloric acid concentration employed by Casares and Casares, who used 1 cc. of the concentrated (approximately 12 N) hydrochloric acid to 100 cc. of the sample. As previously mentioned, their lowest standard contained 0.08 mg. fluorine. The results obtained in the present study, however, indicated that by using a hydrochloric acid concentration obtained by adding 5 cc. of 5 N HCl to 50 cc. of water, as little as 0.01 mg. of fluorine in 50 cc., or 0.2 parts per million, could be differentiated from the corresponding control by means of the zirconium-alizarin reagent as here employed.

After some preliminary experiments, the following procedure was adopted and used in estimating the fluorides in the waters listed in the accompanying table. The estimation of fluorides in these waters represents part of the work of a preliminary survey for the purpose of selecting suitable localities for further study, particularly with reference to a possible correlation of the quantities of fluorides in the drinking waters with the observed degrees of mottled enamel. For the latter purpose, it will, of course, be necessary to make more than single determinations in order to be able to allow for possible seasonal variations or variations in fluorine content due to some other factors.

PROCEDURE

To 50 cc. of the sample of water, or a smaller quantity diluted to 50 cc. with distilled water, and to each of 50 cc. of standards containing known amounts of fluoride in water of similar composition as the sample (prepared on the basis of the analysis), there were added 5 cc. of 5 N HCl and 1 cc. of the diluted zirconium-alizarin reagent, mixing well after each addition. All were allowed to stand, at room temperature, over night (about 18 hours) and then the color of the sample was compared, in 50 cc. (tall form) Nessler tubes, with the colors of the fluoride standards. Since the highest concentration of bicarbonate (645.4 p.p.m.) encountered in this series corresponded to only about 0.1 cc. of the 5 N HCl, and since the water used for the standards contained the same quantity as the sample, no additional HCl was added to balance the alkilinity of the waters.

The diluted zirconium-alizarin reagent was prepared by diluting 1 volume of the more concentrated with 2 volumes of distilled water. The latter was prepared by mixing equal volumes of 0.5-percent aqueous solution of zirconium oxychloride ($ZrOCl_2.8H_2O$) and 0.1-percent aqueous solution of alizarin sodium monosulphonate (Harmer Laboratories Co., Philadelphia). It was found advantageous to add the alizarin solution slowly to the zirconium solution rather than in the reverse order, since when the zirconium solution was added to the alizarin solution, it was often necessary to let the mixture stand much longer (about 18 hours) before it became entirely clear. Although the reagent appears to be fairly stable when kept in a cool dark closet, it was generally used before it was more than a few days old.

Erlemeyer flasks of about 125 cc. capacity were found convenient in this connection. It is preferable, however, to transfer the mixed solutions to the Nessler tubes as soon as practicable. The solutions should also be mixed well immediately before the colorimetric comparisons, since there appears a tendency for the coloring matter to precipitate out on standing.

It was found possible, in some cases, to obtain fair readings after the treated fluoride solutions were allowed to stand at room temperature for only about 4 hours. It appears advantageous, however, to use the longer (over night) standing time, especially since the work can often be arranged so as to make the latter more convenient.

The fluoride standards were prepared from sodium fluoride, the stock solution usually employed containing a quantity of sodium fluoride equivalent to 0.05 mg. fluorine per cubic centimeter. The fluorine quantities of the standards were 0, 0.01, 0.02, 0.03, 0.04, 0.045, 0.05, and 0.055 mg., respectively. It was intended to take a quantity of the sample which would make its fluorine content about 0.04 to 0.05 mg. It appears that the reagent can be utilized most advantageously when the fluorine content is of a magnitude which will cause most of the reddish color to fade out. Under the present conditions this occurred when it was about 0.04 to 0.05 mg. Under these conditions the results obtained were within approximately 15 percent of the theory, which, for some practical purpose, are probably sufficiently close and useful, at least in preliminary surveys.

The samples of water listed in the table, the fluoride contents of which were estimated by the procedure here described, were collected mostly by Dental Surg. H. T. Dean in connection with field studies of mottled enamel.

SUMMARY

A procedure is described for estimating approximately small amounts of fluorides in drinking water, which utilizes the zirconium-alizarin reagent for fluoride. The results obtained in the analyses of 20 samples of water from various localities are reported.

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Analysis of waters containing fluorides

[In parts per million]

Description and source of sample	Residue on evaporation	Loss on ignition	Fixed residue	Silica (SiO_2)	Phosphate (PO_4)	Iron (Fe)	Aluminum (Al)	Calcium (Ca)	Magnesium (Mg)	Sodium and potassium (calculated as Na)	Sodium (Na)	Potassium (K)	Carbonate (CO_3)	Bicarbonate HCO_3	Sulphate (SO_4)	Nitrate (NO_3)	Chloride (Cl)	Fluoride (F)
Spring water, Twomey Springs, Tenn.	154.0	41.0	113.0	16.0	0	0.10	0.04	38.6	3.7	6.3	......	......	10.2	96.4	19.7	0.2	4.0	0.2
Spring water, Mount Pleasant, Tenn.	210.5	68.5	142.0	14.5	1.8	.10	.04	48.6	3.7	6.3	......	......	7.2	80.5	36.2	24.2	11.5	.4
Well water, Fort Stanton, N.Mex.	363.5	64.5	299.0	15.8	0	.05	.23	69.9	12.5	22.9	......	......	......	133.6	139.9	.4	17.5	.6
Water from pipe line at Fort Stanton, N.Mex.	168.0	32.0	136.0	24.0	0	.15	.41	31.2	1.8	15.3	......	......	......	71.4	45.6	1.2	7.0	.6
Cistern water (O'Neil), Rosiclare, Ill.	68.0	13.0	55.0	2.0	0	.15	1.08	19.3	1.6	3.7	......	......	......	52.5	10.5	1.1	1.8	.6
Well water, Rosiclare, Ill.	598.0	106.0	492.0	27.6	0	.24	.88	141.5	33.1	20.0	......	......	......	468.5	80.6	21.5	36.0	.6
Cistern water (near mine), Rosiclare, Ill.	110.6	31.6	79.0	11.4	0	.15	1.21	30.7	1.7	2.8	......	......	15.6	50.0	14.4	.9	1.5	.7
Water 420 feet below ground level at Mexico, Ky.	352.5	90.2	262.3	12.0	0	.25	1.28	77.2	21.8	8.7	......	......	......	270.8	74.9	.2	5.0	1.2
Spring water, near Cave in Rocks, Ill.	314.8	65.0	249.8	16.0	0.9	.20	1.17	97.9	6.7	8.3	......	......	......	306.2	29.8	.6	5.5	1.5
Municipal water, Monmouth, Ill.	1,061.2	89.6	971.6	46.2	0	.10	.45	66.8	30.4	242.9	......	......		288.5	402.8	3.0	118.0	2.0
Municipal water, Fairbury, Ill.	1,388.0	30.5	1,357.5	10.8	0	.20	.05	43.6	17.9	......	435.9	50.3	10.8	392.8	76.9	.9	555.3	2.5
Municipal water, Windsor, N.C.	721.8	9.8	712.0	13.5	0	.15	.15	1.4	1.6	275.6	......	......	18.6	374.5	71.8	.9	134.3	2.6
Municipal water, Minonk, Ill.	1,919.2	31.7	1,887.5	8.3	0	.13	.10	49.0	15.7	......	650.7	42.5	......	414.8	173.2	.4	790.8	2.8
Municipal water, Amarillo, Tex.	419.4	86.9	332.5	58.0	0	.05	.60	47.9	45.1	32.7	......	......	......	358.0	48.9	2.8	9.8	3.5
Municipal water, Myrtle Beach, S.C.	796.0	9.0	787.0	29.4	0	.10	.10	3.4	2.4	317.1	......	......	20.4	645.4	6.6	.8	86.5	4.2
Well water (Oliver), near Conway, S.C.	632.0	8.7	623.3	6.8	0	.05	.09	3.2	2.2	255.6	......	......	14.4	575.8	4.9	.9	45.5	4.5
Well water (Procter), near Conway, S.C.	635.5	3.8	631.7	8.8	1.3	.05	.08	3.6	4.3	255.1	......	......	15.6	585.0	8.7	.8	44.3	4.5
Well water (Edwards), Courtland, Va.	245.8	9.8	236.0	27.4	3.2	.10	.03	.6	.7	85.7	......	......	......	176.9	13.9	.3	16.3	5.0
Well water (Yates-Williams), Courtland, Va.	304.1	15.1	289.0	19.9	2.8	.08	.04	1.2	.8	112.3	......	......	......	228.8	13.4	.5	23.3	6.0
Municipal water, Marion, Ky.	1,152.0	148.0	1,004.0	15.0	0	.05	2.58	215.8	40.1	59.1	......	......	......	225.7	571.8	.1	46.5	20.0

MICRODETERMINATION OF FLUORINE BY THORIUM NITRATE TITRATION

F. J. McClure

The Willard and Winter method for determining fluorine (*11*) with the use of sodium alizarin sulfonate indicator as proposed by Armstrong (*2*) has been modified recently as follows: by Hoskins and Ferris (*8*) who introduced the use of a buffer of monochloroacetic acid; by Armstrong (*1*) who titrated fluoride in an aqueous solution rather than in an alcoholic solution, and used silver perchlorate to remove interfering chlorides; and by Churchill, Bridges, and Rowley (*3*) who eliminated the effect of phosphate by a double distillation. Rowley and Churchill (*10*) applied the aqueous titration to the determination of quantities of 1 to 50 mg. of fluorine. Eberz, Lamb, and Lachele (*6*) studied the titration in alcoholic solution of quantities of 100 to 150 micrograms of flourine. [Dahle and associates (*5*) have recently studied a "back-titration procedure" as suggested to them by W. S. Allen.]

The microdetermination of fluorine according to Armstrong gave an average error of −2.0 percent in the recovery of 2.0 micrograms of fluorine (*1*) and "the lower limit seems to be in the vicinity of 0.4 gamma." The method (*1*) appears particularly advantageous also in that the quantities of reagents and the analytical sample may be reduced to a minimum. The results presented herein, however, show that the titration is less sensitive than Armstrong reports. One of the most disturbing features is an appreciable blank titration which tends to fix the minimum quantity of total determinable fluorine appreciably higher than Armstrong suggests. The minimum quantity actually titrated in aliquots, with reasonable accuracy, was found to equal about 5.0 micrograms.

LIMIT OF ACCURACY OF THE TITRATION

Titration results with pure fluoride solutions using Armstrong's method, with the use of the Hoskins and Ferris buffer, are given in table I. The determination of the end point was found to be difficult and frequently uncertain. For best results the color of the unknown and standard was judged with the vial resting on a dull white surface, in light produced by a monochromatic daylight bulb. The end point was scarcely sensitive to a variation of less than 0.030 to 0.050 cc. of the thorium nitrate, equivalent to 0.15 to 0.25 microgram of fluorine. In order to obtain the high recovery which Armstrong reports, it is necessary to titrate much closer than this—i.e., with an error equal to about 0.04 microgram of total fluorine (*1*) or even less, since aliquots of the total are titrated.

As shown in table I there is an average blank titration of 0.355 cc. which represents thorium nitrate required to produce the pink colored lake formed following the fluorine-thorium nitrate reaction and used as the end point. It was not thought desirable to have this titration blank equal to more than a third of the total actual titration figure. This in itself restricts the method to the determination of a minimum of about 10.0 micrograms of total fluorine under the conditions that the aliquot titrated is not less than ⅕—i.e., at least 2 micrograms are taken for the actual titration, and 3

Table I.—*Titration of aqueous fluoride solutions with thorium nitrate*

Fluorine added	$Th(NO_3)_4$ required for 1/10 aliquot[a]	Total fluorine titrated	Total fluorine found	Error in fluorine found	Recovery
Gamma	*Cc.*	*Gamma*	*Gamma*	*Gamma*	*Percent*
10.0	0.200	1.04	10.4	+0.4	104.0
10.0	0.247	1.28	12.8	+2.8	128.0
11.3	0.232	1.21	12.1	+0.8	107.1
11.3	0.216	1.13	11.3	0.0	100.0
20.0	0.369	1.92	19.2	−0.8	96.0
20.0	0.408	2.12	21.2	+1.2	106.0
50.0	1.038	5.40	54.0	+4.0	108.0
50.0	1.000	5.20	52.0	+2.0	104.0
68.0	1.354	7.04	70.4	+2.4	103.5
68.0	1.342	6.98	69.8	+1.8	102.6
100.0	1.940	10.09	100.9	+0.9	100.9
100.0	1.831	9.52	95.2	−4.8	95.2
100.0	1.873	9.74	97.4	−2.6	97.4

[a] Net titration after subtracting blank. Titration figures for five blanks equaled 0.303, 0.363, 0.313, 0.358, and 0.360 cc.; av., 0.355 cc. 1 cc. of $Th(NO_3)_4$ = 5.2 gamma of fluorine.

or 4 aliquots are titrated to obtain an average titration figure.

For best results the actual titration was carried out on at least 5 to 19 micrograms of fluorine, requiring from 1.5 to 3.0 cc. of the thorium nitrate. These limits are more in keeping with the limits of accuracy found in the actual titration and with the size of the blank titration figure.

EFFECTS OF EVAPORATION IN GLASS OR PORCELAIN

Table II gives results obtained from evaporation on a water bath of pure fluoride solutions, using glass, porcelain, and platinum containers. The recoveries are generally low with evaporation in glass and porcelain. The addition of the buffer did not give the characteristic bright yellow color to these solutions. On adding thorium nitrate the pink color of the end point appeared quite as usual but the fluoride-thorium nitrate reaction apparently was interfered with in view of the low titration figures. These results show the need of avoiding the use of porcelain and glass containers for the evaporation of the alkaline fluoride distillates. The cause of this interference was not determined. It indicates strikingly the extreme sensitivity of this titration to slightly modified conditions.

ADDITION OF SILVER SULFATE TO DISTILLING FLASK

Hoskins and Ferris (*8*) reported an effect of chloride on the alcoholic titration; Armstrong (*1*) found chloride to interfere in both aqueous and alcoholic titration of fluorine. The author's data show a similar interference beginning at about 4.0 mg. of chlorine per cc.

Armstrong (*1*) eliminated chloride from the distillate by a rather involved procedure requiring precipitation with silver perchlorate, filtering, redistilling the filtrate, and evaporating the second distillate which is treated with activated charcoal and filtered again before making up to volume. Eberz, Lamb, and Lachele (*6*) add silver perchlorate solution to a solution of the ash to be distilled, carefully avoiding any excess of silver salt. This ash mixture is dried and transferred to the distilling flask. They found distillation in the presence of silver chloride precipitate "entirely satisfactory, not giving trouble by bumping, holding back fluorine, or releasing the chloride which it is desired to eliminate" (*6*).

The precipitation of chloride in the distilling flask as the silver salt recommends itself as a simple and effective procedure and had been studied prior to the appearance of the article by Eberz et al. (*6*). The recovery of fluorine was satisfactory (table III). Although bumping at first proved troublesome, this was partially overcome by the use of solid silver sulfate added directly to the ash solution or to a first distillation concentrate. A less flocculent precipitate was formed under these conditions than when the silver salt was added in a solution. Bumping is eliminated to some extent also in the steam-distillation procedure when the precipitate is kept continuously agitated by steam entering the mixture. It does not appear necessary to avoid a slight excess of silver sulfate. If a second distillation is to be made the required silver sulfate is added for the second distillation.

APPLICATION OF METHOD TO BIOLOGICAL MATERIALS

As has been pointed out by Dahle and Wichmann (*4*) and by Hoffman and

Table II.—*Comparison of glass, porcelain, and platinum containers for evaporation of alkaline solutions of sodium fluoride*

(150-cc. volumes evaporated, made to 10 cc., 1/10 aliquot titrated)

Fluorine added	Glass			Porcelain			Platinum		
	$Th(NO_3)_4$ required for 1/10 aliquot	Error in total fluorine	Recovery	$Th(NO_3)_4$ required for 1/10 aliquot	Error in total fluorine	Recovery	$Th(NO_3)_4$ required for 1/10 aliquot	Error in total fluorine	Recovery
Gamma	*Cc.*	*Gamma*	*Percent*	*Cc.*	*Gamma*	*Percent*	*Cc.*	*Gamma*	*Percent*
0.0	0.340	....	..	0.319	....	...	0.385	...	...
0.0	0.319	....	..	0.335	....	...	0.356	...	...
0.0	0.358	....	..	0.329	....	...	0.413	...	...
10.0	0.470	−5.4	54.0	0.531	+0.2	102.0	0.626	+1.9	119.0
10.0	0.413	−6.3	37.0	0.486	−2.1	79.9	0.572	−0.7	93.0
50.0	1.125	−10.7	78.6	1.239	−4.5	91.0	1.309	−3.7	92.6
50.0	1.098	−12.0	76.0	1.138	−9.5	81.0	1.450	+2.3	104.6
100.0	2.226	−5.7	94.3	1.728	−29.9	70.1	2.416	+1.7	101.7
100.0	2.188	−12.6	87.4	2.114	−10.7	89.3	2.410	+1.3	101.3
150.0	3.300	−2.0	98.6	2.992	−16.8	88.8	3.377	−0.3	99.8
150.0	3.110	−10.6	92.9	3.084	−12.2	91.8	3.441	+2.9	101.9

Lundell (*7*), considerable uncertainty may still surround the method of separation of fluorine by volatilization in the presence of perchloric or sulfuric acid. It is desirable, therefore, to report satisfactory results (average error about ±3.0 micrograms) using perchloric acid and the general technique of Armstrong (*1*) and of Hoskins and Ferris (*8*), in the recovery of fluoride added to the distilling flask. The distilling blank titration equals 0.400 to 0.500 cc. (these figures include titration blank of 0.20 to 0.30 cc. previously discussed), owing perhaps to fluorine in the reagents or to volatilized perchloric acid. It was not found possible to change this blank appreciably by collecting several volumes of distillate from the same sample of perchloric acid or by heating the acid to 170° C. and then collecting 150-cc. samples of distillate at 140° C. Perchlorate ion was found to affect the titration when equal to about 2.0 to 4.0 mg. per cc.

As applied to the determination of the fluorine in biological material, the total body of experimental test rats, for example (*9*), the procedure was as follows:

The animal body was autoclaved at 120° C. (15 pounds) for about 0.5 hour, dried first in a warm air bath and then in an oven at 100°, moistened with 5 per cent magnesium acetate (*12*), and ashed in a muffle furnace at 500° C. An aliquot of the ash, equaling approximately 0.50 gram or less, was taken for the fluorine determination.

The ash sample was placed in a 50-cc. Classen flask together with 5 cc. of 60 per cent perchloric acid, 0.1 gram of pure silica, 10 cc. of distilled water, and two glass beads. The mixture was steam-distilled at a temperature of 138° to 142° C. receiving the distillate in a solution kept definitely alkaline to phenolphthalein, and continuing the distillation until 150 cc. were collected. The distillation should be completed in about 30 minutes. The alkaline distillate was placed in a platinum dish, evaporated on the water bath to about 5 cc., neutralized with dilute hydrochloric acid, and made up to 10 cc. Four or five 1-cc. aliquots were titrated in a manner essentially as described by Armstrong (*1*). Some results obtained in applying the above procedure to bone and body ash are given in table IV.

Table III.—*Elimination of effect of chloride by adding of silver sulfate*

(Sodium chloride and silver sulfate added to distilling flask, 150-cc. distillate collected, evaporated, made to 5 cc., 1/5 aliquot titrated)

Fluorine added	NaCl added	Ag_2SO_4 added	$Th(NO_3)_4$ required for 1/5 aliquot	Total fluorine found	Error in fluorine found	Recovery
Gamma	*Gram*	*Grams*	*Cc.*	*Gamma*	*Gamma*	*Percent*
20.0	0.0	0.0	1.228	20.7	+0.7	103.5
20.0	0.0	0.0	1.341	24.0	+4.0	120.0
20.0	0.0	0.0	1.499	21.0	+1.0	105.0
20.0	0.0	0.0	1.396	19.1	−0.8	95.5
20.0	0.1	0.0	1.660	25.0	+5.0	125.0
20.0	0.1	0.0	1.680	25.4	+5.4	127.0
20.0	0.2	0.0	1.725	26.5	+6.5	127.5
20.0	0.2	0.0	1.718	26.3	+6.3	126.5
40.0	0.4	0.0	2.324	46.5	+6.5	116.2
40.0	0.4	0.0	2.456	49.8	+9.8	124.5
20.0	0.1	0.3	1.358	23.3	+3.3	116.5
20.0	0.2	0.6	1.343	21.2	+1.2	106.0
25.0	0.2	0.6	1.364	22.5	+2.5	110.0
50.0	0.2	0.6	2.405	48.6	−1.4	97.2
50.0	0.2	0.6	2.525	51.6	+1.6	103.2
100.0	0.4	1.2	2.421	97.8	−2.2	97.8
100.0	0.4	1.2	2.529	103.3	+3.3	103.3

Taple IV.—*Analysis of bone ash*

Sample weight	Fluorine added	$Th(NO_3)_4$ required for aliquot	Total fluorine found	Recovery of added fluorine	Fluorine in sample
Gram	*Gamma*	*Cc.*	*Gamma*	*Gamma*	*Percent*
0.500	0.0	1.729	59.5	..	0.0119
0.500	0.0	1.695	60.6	..	0.0121
0.500	50.0	2.632	108.4	48.4	0.0120
0.500	50.0	2.596	106.6	46.6	0.0120
0.500	50.0	2.661	109.9	49.9	0.0120
0.040	0.0	2.108	77.3	..	0.1932
0.040	0.0	2.181	80.7	..	0.2017
0.040	0.0	2.735	92.5	..	0.2312
0.040	0.0	2.552	98.1	..	0.2452
0.020	0.0	2.915	100.0	..	0.5000
0.020	0.0	2.674	103.8	..	0.5190
0.020	0.0	3.215	112.7	..	0.5635
0.020	0.0	3.246	114.0	..	0.5200

In the majority of these determinations 50.0 to 100.0 micrograms were adopted as the range of determinable fluorine and 0.50 gram or less of ash constituted the analytical sample. It was intended to keep the size of the analytical sample as small as possible to avoid interfering elements. The recovery of fluorine in the presence of these small quantities of ash was within the limits of error found for pure fluoride solutions added to the distilling flask. Duplicate determinations agreed within 2.0 to 6.0 micrograms of fluorine. In bone and tooth ash or total body ash the only interfering substance would appear to be phosphate. Its effect was eliminated by a double distillation, as proposed by Churchill et al. (*3*) when the sample exceeded 0.1 gram. [An interference of phosphate (PO_4) on the titration was noted at a concentration of about 1.0 microgram per cc.]

In the determination of fluorine in foods and biological materials where the quantities of fluorine present may be 10 p.p.m. or less, more serious difficulties are encountered. The size of sample which will yield a determinable quantity of total fluorine may be extremely large and may include a large yield of ash containing large quantities of interfering chlorides and phosphates, and perhaps other interfering agents. An effort was made to apply the method outlined to dried milk powder which is extremely low in fluorine and high in total ash containing considerable phosphate and chloride. In limiting the determinable fluorine to a minimum of 50 micrograms, at least 100 grams of a material containing 0.5 to 1.0 p.p.m. of fluorine must constitute the analytical sample. Total ash from 100 grams of milk powder is excessive for a 50-cc. flask and 5 cc. of perchloric acid are not enough to keep the ash in solution for distilling. Even on reducing the sample to 50 grams as was done for the data reported in table V, 10 cc. of perchloric acid, instead of the recommended 5 cc., were required. Under these modified conditions 50-gram duplicate samples varied as much as 14 micrograms of fluorine in an average of approximately 30

TABLE V.—*Analysis of skim-milk powder*

Sample weight	$Mg(Ac)_2$	Ag_2SO_4	Fluorine added	Aliquot titrated	$Th(NO_3)_4$ required for aliquot	Total fluorine found	Recovery of added fluorine	Fluorine in sample	
Grams	*Gram*	*Grams*	*Gamma*		*Cc.*	*Gamma*	*Gamma*	*Gamma*	*P.p.m.*
0.0	0.0	0.0	0.0	1/5	0.453	. . .	. . .	. .	. .
0.0	0.0	0.0	0.0	1/5	0.403	. . .	. . .	. .	. .
0.0	0.0	0.0	0.0	1/5	0.383	. . .	. . .	. .	. .
0.0	0.0	0.0	0.0	1/5	0.471	. . .	. . .	. .	. .
0.0	0.5	2.0	0.0	1/5	0.643	8.9	. . .	. .	. .
0.0	0.5	2.0	0.0	1/5	0.623	8.2	. . .	. .	. .
0.0	0.5	2.0	100.0	1/10	2.614	108.6	100.1	. .	. .
0.0	0.5	2.0	100.0	1/10	2.586	107.2	98.7	. .	. .
0.0	0.5	2.0	50.0	1/5	2.405	57.1	48.6	. .	. .
0.0	0.5	2.0	50.0	1/5	2.525	60.1	51.6	. .	. .
50.0	0.5	2.0	. . .	1/5	2.256	45.4	. . .	36.9	0.73
50.0	0.5	2.0	. . .	1/5	1.668	30.6	. . .	22.1	0.44
50.0	0.5	2.0	. . .	1/5	1.929	37.1	. . .	28.6	0.57
50.0	0.5	2.0	100.0	1/10	3.694	162.6	124.9	(29.2)	. .
50.0	0.5	2.0	100.0	1/10	2.840	111.0	81.3	(29.2)	. .
50.0	0.5	2.0	50.0	1/10	2.453	100.6	62.9	(29.2)	. .
50.0	0.5	2.0	50.0	1/10	2.146	85.2	47.5	(29.2)	. .

micrograms of total fluorine determined. The results for the same sample showed 0.44 to 0.73 p.p.m. of fluorine present. Recovery of 50 to 100 micrograms of added fluorine varied from 80.0 to 125.0 percent. Doubtless the quantity of fluorine dealt with is too small, but a larger analytical sample would require increased quantities of reagents, a larger flask, and perhaps other modifications, which may affect the distillation (*5*) and alter the titration conditions.

No attempt has been made to introduce such modifications as appear to be necessary from the above results, in order to apply the method successfully to substances such as milk powders.

SUMMARY

The microdetermination of fluorine by titration in aqueous solution with thorium nitrate according to Armstrong's latest technique did not give the high degree of accuracy obtained by Armstrong. The recovery of fluoride added to bone ash in 50.0 to 100.0-microgram quantities varied about ±5.0 micrograms from the total added. Duplicate samples of bone and tooth ash agreed within 2.0 to 6.0 micrograms of total fluorine, where a total of 50.0 to 100.0 micrograms of fluorine were determined, the size sample equaling not more than 0.5 gram of ash. An attempt to apply the method to the analysis of a material such as milk powder containing less than 1.0 p.p.m. of fluorine was not successful.

The necessity of avoiding glass or porcelain containers in evaporation of alkaline fluoride distillates, previous to titration with thorium nitrate, is indicated. A method for the prevention of volatilization of chloride, by precipitation with silver sulfate added to the distilling flask, was found satisfactory.

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MODIFIED ZIRCONIUM-ALIZARIN REAGENT FOR DETERMINATION OF FLUORIDE IN WATER

By STEPHEN MEGREGIAN and FRANZ J. MAIER

The fluoridation of public water supplies for reducing the incidence of dental caries has created a need for a simple, accurate method for making routine determinations of fluoride concentration in water. An accurate determination can be made by a controlled distillation process which is time-consuming and difficult (*1*). Many direct colorimetric methods have been developed to estimate fluorides in water. The more accepted ones are based on the reaction of zirconium and alizarin to produce a lake which is decolorized by the fluoride ion.

The present standard method (*1*) using the zirconium-alizarin color reaction was developed by several investigators, beginning with DeBoer (*2*), and followed by Elvove (*3*), Sanchis (*4*), and Scott (*5*, *6*). The evolution of the method has been characterized by changes in the reaction conditions and acid concentrations. None of the investigators except DeBoer published the experimental basis for recommended ratios of zirconium to alizarin. Although the reagent was improved considerably in its ability to reduce sulfate and chloride interferences and to shorten the reaction time, some of the principal shortcomings of the reaction, such as nonconformity to the color laws, limited effective fluoride range, and little color change per increment of fluoride, were not significantly improved or eliminated. Todd (*7*) and Taras (*8*) applied the standard method to spectrophotometric study and pointed out these deficiencies.

EXPERIMENTAL PROCEDURE

The purpose of the investigation described here was to improve the standard method for fluorides by modifying the reagent. Except when visual methods were employed, color comparisons were made with a Beckman Model B spectrophotometer. Most of the observations were made using a 70-mm. special cell in order to utilize the longest practical light path with this instrument.

Preliminary experiments revealed that when the ratio of alizarin to zirconium was greater than required by the standard method, there was considerable increase in the total color developed and a greater color change per unit of fluoride. Figure 1 shows the effect of alizarin concentration on the color produced in the zirconium-alizarin reaction. The readings were taken after the reagent, prepared with Scott's mixed acid, was allowed to react for 60 minutes at room temperature with distilled water in the same manner as in a standard fluoride determination. Scott's mixed acid is prepared by diluting 37 ml. concentrated H_2SO_4 to 500 ml., 112 ml. concentrated HCl to 500 ml. and mixing to make 1 liter. To prepare the final reagent 900 ml. of the mixture is used. In the above studies, the alizarin was always made up and kept as a separate solution and the zirconium was made up with the appropriate amount of mixed acid. By keeping the reactants separate, aging errors are eliminated.

The color increment per unit of fluoride for each of the various ratios of alizarin to zirconium was next determined and plotted as indicated in figure 2. From these curves it is evident that the ratio 7.5 to 1.0 by weight gives the greatest color increment per unit of fluoride, provides a satisfactory range of fluoride concentration, and produces a standard curve which follows Beer's law. Increasing or decreasing the concentration of zirconium and alizarin in the reagent has the effect of increasing or decreasing the total color production without any appreciable change in the absorbency per unit of fluoride. Higher fluoride concentrations (more than 4.0 p.p.m.) can be determined by increasing the amounts of zirconium and alizarin in the reagent.

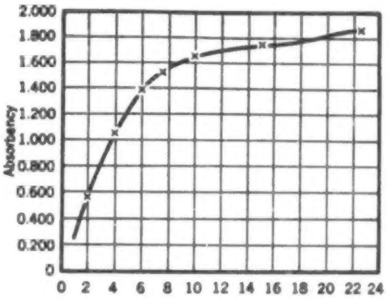

FIGURE 1.—*Effect of alizarin concentration on color production of the zirconium-alizarin reagent*

Zirconium content was 0.5 mg. per 100 ml.; reaction time, 60 minutes; light path, 7 cm.; and wave length, 525 mμ.

In Boonstra's modification of the standard method (*9*), an alizarin-zirconium ratio of 22.5 to 1.0 by weight is recommended. The curve shown in figure 2 indicates that this ratio falls below the maximum sensitivity for fluorides.

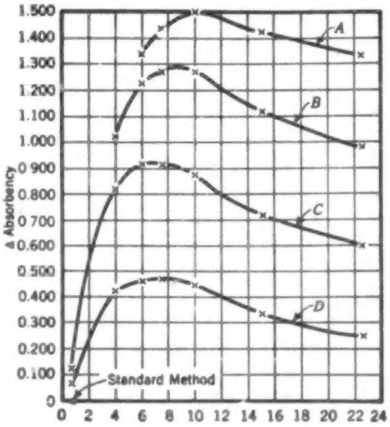

FIGURE 2.—*Change in absorbency per unit fluoride for various weight ratios of alizarin to zirconium*

The zirconium content was 0.5 mg. per 100 ml.; reaction time, 60 minutes; light path, 7 cm.; and wave length, 525 mμ. The fluoride increments were: for Curve A, 0.0–4.0 p.p.m.; for Curve B, 0.0–3.0 p.p.m.; for Curve C, 0.0–2.0 p.p.m.; for Curve D, 0.0–1.0 p.p.m.

On the basis of these observations, the following method was selected as most suitable for the spectrophotometric determination of fluoride in water. The extinction per centimeter of light path per part per million fluoride in the range of 0 to 3.0 p.p.m. fluoride is approximately 0.070, thus making it possible to determine fluoride residuals accurately to 0.10 p.p.m. using 1-cm. cuvettes and to make readings of 0.02 p.p.m. easily with cells of longer light path.

REAGENTS

A. 0.750 g alizarin red S dissolved in 1 liter distilled water.

B. 0.368 g $ZrOCl_2 \cdot 8H_2O$ or 0.294 g $ZrO(NO_3)_2 \cdot 2H_2O$ dissolved in 100 ml distilled water and 900 ml. Scott's mixed acid added to make 1 liter.

DETERMINATION

Add 5 ml. only A followed by 5 ml. B. accurately measured to 100 ml. of sample or aliquot, the fluoride content of which is below 4.0 p.p.m. Mix after each addition. Read at 525 mμ after allowing to stand for 60 minutes at the standard temperature of the spectrophotometric curve.

In the construction of this curve, a series of fluoride standards between 0.00 and 4.00 p.p.m. are prepared and allowed to react with the reagent for 60 minutes. The spectrophotometer is set to zero absorbency with distilled water in the absorption cell. Successive readings of the prepared standards are then taken and plotted. Because this color reaction varies with time, it is impracticable to set the zero point with a reagent blank (a standard containing 0.00 p.p.m. fluoride) when a direct-reading spectrophotometer (similar to Beckman Model B or DU) is used. This restriction does not apply to instruments operating on the split-beam (null point) principle, provided the instrument is not subject to drift.

It is recommended that a new standard curve be constructed for each fresh

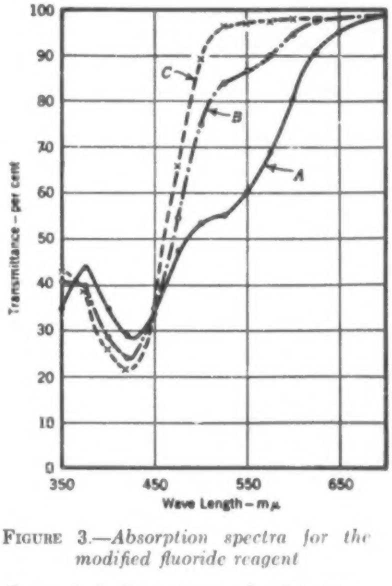

FIGURE 3.—*Absorption spectra for the modified fluoride reagent*

Curve A is for reagent plus 0.0 p.p.m. fluoride; Curve B. for reagent plus 3.0 p.p.m. fluoride; Curve C, for alizarin red S. plus mixed acid. Light path was 1 cm.

batch of reagent prepared, as slight errors in weighing or in the purity of the chemicals used will produce a significant error in the results. Figures 3 and 4 give the absorption spectrum and a standard curve for the above reagent.

INTERFERENCES

The study of interferences was confined to ions normally present in natural water or added to drinking water during treatment. With the exception of aluminum, the ions did not in any test introduce a greater error than the present standard method and usually the interfering effects were greatly reduced. Figure 5 shows the extent of interference by the various ions. Calcium up to 400 p.p.m. and magnesium up to 200 p.p.m. have no effect. Sulfate and hexametaphosphate react similarly to fluoride, reducing the color—400 p.p.m. SO_4^{-2} being equivalent to 0.1 p.p.m. fluoride and 1.1 p.p.m. $(NaPO_3)_6$ being equivalent to 0.1 p.p.m. fluoride. Chloride, bicarbonate (alkalinity × 1.22), and ferric iron intensify the color produced by the reagent and thus produce an effect opposite that of fluoride—1,800 p.p.m. Cl^{-1}, 400 p.p.m. HCO_3^{-1} and 5 p.p.m. Fe^{+3} each reducing the fluoride reading by 0.1 p.p.m.

Chlorine interferes by exerting a bleaching action on alizarin red S. The extent of its interference depends on both the amount of chlorine and the amount of fluoride present; therefore, reduction of chlorine is necessary before the fluoride determination can be made. The effect of chlorine is shown in table 1.

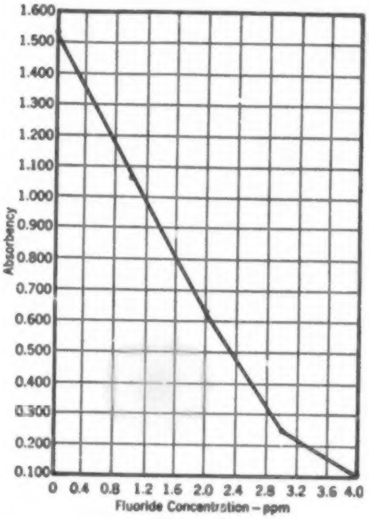

FIGURE 4.—*Standard curve for the modified fluoride reagent*

Light path was 7 cm; reaction time, 60 minutes; wave length, 525 mμ. Zirconium content was 0.5 mg. in 100 ml. water and ratio of alizarin to zirconium was 7.5 to 1 by weight.

TABLE 1.—*Interference by chlorine*

Chlorine Present	Apparent F when 0 ppm. F is present	Apparent F when 1.0 ppm. F is present
ppm.	*ppm.*	*ppm.*
0.5	0.00	1.06
1.0	0.07	1.10
2.0	0.20	1.27
3.0	0.36	1.42
5.0	0.78	1.70

Arsenite, sulfite and thiosulfate were used as dechlorinating agents. Sodium arsenite and sodium sulfite up to 200 p.p.m. in excess did not interfere with the subsequent determination. Sodium thiosulfate in concentrations more than 100 p.p.m. excess interferes because of its tendency to precipitate sulfur in acid solutions.

Phosphate also reacts similarly to fluoride. Its behavior, however, is unique in that when fluoride is absent, phosphate exerts a much greater effect on the reagent than when fluoride is present. When no fluoride is present, 1.5 p.p.m. PO_4^{-3} gives a result equivalent to that of 0.1 p.p.m. fluoride. When 1.0 p.p.m. fluoride is present, 5.0 p.p.m. PO_4^{-3} produces no interference, but 10 p.p.m. PO_4^{-3} will produce an interference equivalent to 0.4 p.p.m. fluoride. When 2.0 p.p.m. fluoride is present, 10 p.p.m. PO_4^{-3} produces no interference, but 20 p.p.m. PO_4^{-3} will be equivalent to 0.32 p.p.m. fluoride. The explanation of this phenomenon is not known. It is possible that the fluoride and phosphate ions, under the acidic condition of the test, combine to form a single anion, $(PO_3F)^{-2}$, which is also capable of decolorizing the zirconium-alizarin lake. A correction curve for phosphate is shown in figure 6.

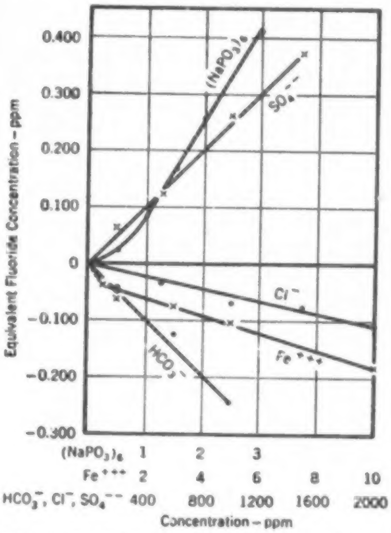

FIGURE 5.—*Correction curves for ions interfering with the fluoride determination*

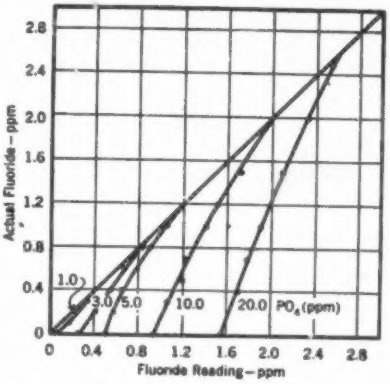

FIGURE 6.—*Correction curves for phosphate interference*

Aluminum ion exerts an inhibitory effect on the fluoride reaction. It forms a complex with fluoride ions $(AlF_6)^{-3}$ and prevents them from reacting with the zirconium-alizarin lake. Furthermore, the action of a given concentration of aluminum on the fluoride reaction is increased as the concentration of fluoride is increased. When there are no fluoride ions present, aluminum up to 5.0 p.p.m. does not interfere. When the fluoride concentration is 1.0 p.p.m., 1.0 p.p.m. aluminum reduces the fluoride reading 0.39 p.p.m.; 2.0 p.p.m. reduces it 0.57 p.p.m.; and 3.0 p.p.m. reduces it 0.63 p.p.m. When the fluoride concentration is 2.0 p.p.m., 1.0 p.p.m. aluminum reduces it 0.47 p.p.m.; 2.0 p.p.m. reduces it 0.86 p.p.m.; and 3.0 p.p.m. reduces it 1.13 p.p.m. A correction curve for aluminum is shown in figure 7.

It is evident from the above data that phosphates, aluminum and chlorine can cause majors errors in the fluoride determination if their presence or concentration is not known. Chlorine should cause no great concern to the analyst because it can easily be determined and eliminated. The presence of significant amounts of aluminum and phosphate in most public water supplies is rare. It should be required, however, that the

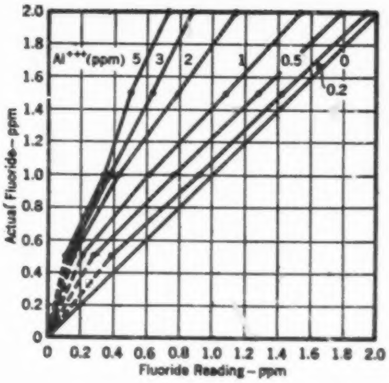

FIGURE 7.—*Correction curves for aluminum interference*

analyst performing the fluoride test on an unknown water also make phosphate and aluminum determinations or a check determination by distillation before accepting as accurate the results of the spectrophotometric test.

DISCUSSION

From the data presented, it is evident that the recommended reagent of itself need not be the one most practical for all laboratories. By holding the alizarin-zirconium ratio in the neighborhood of 7.5 to 1.0 by weight and varying the concentration of the reactants, it is possible to adjust the reagent to the fluoride range that is encountered most frequently in routine work. A change of 20 percent in the zirconium-alizarin concentration in the reagent will move the absorbency reading of the spectrophotometer approximately 0.070 for each centimeter of light path available, but will have very little effect on the fluoride increment. Major changes in the alizarin-zirconium ratio will produce no practical benefits under the specified conditions of the reaction.

The effect of temperature on the standard curve was not studied, but it is obvious from previous work cited that the temperature of the unknown must approximate the temperature at which the standard curve was prepared. It would be advantageous for the analyst performing a large number of fluoride determinations by the above method to prepare a series of standard curves depending on the temperatures prevailing in the water laboratory.

The effect of time is important; 60 minutes was chosen as a satisfactory time interval for accurate work. Although the color-producing reaction between zirconium and alizarin continues for an indefinite period of time, the change in the amount of color produced after 60 minutes is such that a 10-minute error in reading will introduce an error of less than 0.05 p.p.m. F. The error is much greater if a shorter reaction time interval is used, and decreases slowly as longer time intervals are applied. Thus, it is of no great advantage to prolong the reaction time, but a shorter reaction time will introduce greater errors in the individual readings. Figure 8 illustrates the effect of time on the amount of color produced by the reagent.

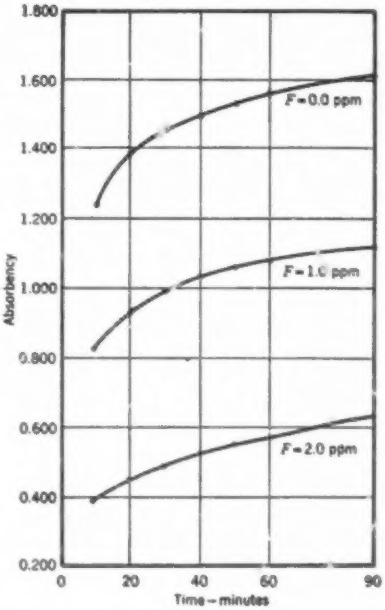

FIGURE 8.—*Rate of color development with recommended spectrophotometric reagent*

Wave length was 525 mμ; temperature 23°C; light path 70 mm.

No effort was made to study in detail the concentration of acid which would be best for this reagent. The acid concentration given by the standard method was accepted as satisfactory. Major changes in acid concentration, such as doubling or halving the recommended amount, will produce significant variations in the color intensity developed by the reagent, and also in color increment per unit of fluoride. Acid concentrations in the magnitude of that recommended above seem to have the best combination of total color production and increment of color per unit of fluoride. Reduction of the acidity will increase color production but will retard the color increment per unit of fluoride, whereas increase of the acidity will retard both.

VISUAL COMPARISON

Many water laboratories that will be required to run routine fluoride determinations do not posses spectrophotometric equipment. Therefore, the above modified reagent was applied to visual comparison technique, using Nessler tubes. The recommended reagent is not completely satisfactory for visual comparison because the color in the low fluoride range is so intense that it becomes difficult to differentiate between fluoride increments 0.1 p.p.m. apart in the range of 0.0 to 1.0 p.p.m. fluoride. To overcome this difficulty the reagent was further modified so that it would be sensitive to small increments of fluoride when observed through Nessler tubes. Many combinations were tried. Evaluation of the several combinations was based on the ability of the eye to differentiate 0.02 p.p.m. fluoride in the neighborhood of 1.0 p.p.m. and also on the ease of reading fluoride concentrations up to 2.0 p.p.m. The reagent recommended below fulfilled these conditions. It should be noted that this reagent can be prepared as a single reagent. It is unnecessary to separate the alizarin and zirconium because the aging effect of the reagent is nullified when a series of standards is prepared simultaneously with the unknown water sample.

REAGENT RECOMMENDED FOR VISUAL COMPARISON TECHNIQUE

1. Dissolve 0.420 g alizarin red S in 100 ml. distilled water.
2. Dissolve 0.20 g $ZrO(NO_3)_2 \cdot 2H_2O$ or 0.251 g $ZrOCl_2 \cdot 8H_2O$ in 100 ml. distilled water.
3. Add 58.6 ml. concentrated H_2SO_4 to 500 ml. distilled water.
4. Mix by pouring (1) into (2), add (3) and make up to 1 liter with distilled water. The reagent can be used after standing 1 hour.

METHOD

Add 5 ml. accurately measured of the reagent to 100-ml. samples and a series of standards; mix. Allow to stand approximately 1 hour before making comparison.

The above reagent is prepared with the acid recommended by Lamar (*10*).

It was found that it is easier by eye to distinguish slight changes in color with this acid than with the one recommended by the standard method because the background of yellow color seems less intense when H_2SO_4 alone is used.

Interference by other ions is practically the same with this reagent as with the one recommended for spectrophotometric measurement, for the alizarin-zirconium ratio is but slightly changed. Phosphate has even less effect, 10 p.p.m. being necessary to produce an apparent fluoride residual of 0.1 p.p.m. when no fluoride is present. There is no interference at 20 p.p.m. phosphate when 1.0 p.p.m. is present.

SUMMARY

A modified zirconium-alizarin reagent is proposed for use in the spectrophotometric determination of fluoride in water. This reagent is sensitive to small increments of fluoride over a range of 0.0 to 3.0 p.p.m. and it approximates Beer's law over this range. The effect of interfering ions is evaluated.

By further modification, the reagent is adjusted for visual comparison techniques so that it is effective over a range of 0.0 to 2.0 p.p.m. fluoride and sensitive to 0.02-p.p.m. increments of fluoride.

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DISCUSSION

STANDARD METHODS SUBCOMMITTEE ON FLUORIDES

Subcommittee S–14 on Fluorides of AWWA Committee E5–9, Standard Methods for the Examination of Water and Sewage. The subcommittee personnel who prepared this discussion include: Charles A. Black, Chairman; W. L. Lamar; and Michael Taras.

The modified zirconium-alizarin reagent for the determination of fluoride in water introduced here presents certain fundamentals for the adaptation of the zirconium-alizarin procedure to photometric methods. As a result of its review of the work of Megregian and Maier on the selection of the proper zirconium-alizarin ratio and the optimum wave length for the use of this ratio, Subcommittee S–14 can report that this method presents a definite contribution in the adaptation of the fluoride determination to photometric procedures.

The method proposed for photometric work seems to follow Beer's law in concentrations up to 3 p.p.m. as this is the recommended limit set by the authors for the particular ratio of zirconium to alizarin used. A paper published by Johanna P. Boonstra on a modification of the Sanchis method (see reference 9, above) also provides a photometric procedure that was studied by this committee and found to be inferior to the Megregian and Maier modification on several significant counts. The 4-hour reaction time of the Boonstra method is a major drawback as is the extra operation of heating the sample. Also, the committee failed to obtain reproducible results consistently with this modification.

In reviewing the article by Megregian and Maier, the different committee members made a number of points which, it was felt, should be presented as suggestions and notations in this discussion:

1. Of particular value in this presentation is the report of the successful use of sodium thiosulfate, sodium sulfite, and sodium arsenite for eliminating the common interferences contributed by free chlorine residuals. Controversy has arisen in some quarters about the applicability of sodium thiosulfate for this purpose. This misunderstanding about sodium thiosulfate presumably stems from the practice of adding macro, or excessive, quantities to remove micro, or trace, quantities of chlorine. The body of knowledge on these matters must be built up by experimental evidence such as is presented here by the authors.

2. The color filter and the initial light intensity are important considerations in the use of a filter photometer for the fluoride determination. If a long cell path, such as 5 cm., is employed, the relatively deep color of the reagent blank coupled with the light intensity will limit the effective transmission spread. Committee member Taras has experience with two types of color filters which illustrate this point. Fortunately, either of these filter types can be inserted in the same instrument. The regular green filter supplied with the instrument is quite thick, making it necessary to null the reagent blank at 20 percent transmittance. The thinner Wratten green filter, on the other hand, passes more light, thereby permitting a null at 50 percent transmittance and affording a transmittance reading up to 87 percent between 0.0 and 1.5 p.p.m. fluoride. The transmittance spread with the thicker filter is between 20 and 48 percent for the same fluoride range. The thickness of the filter and not the wave length is responsible for this phenomena. In the case of the Beckman Model DU spectrophotometer, it is possible to use the extended range of 10 to 80 percent transmittance for a concentration of 0.0 to 1.5 p.p.m. merely by enlarging the slit width to about 1.5 mm., admitting more light, instead of the customary method of operating with minimum slits.

3. It should be emphasized that, if a photometric determination is made with this method, the actual determination of the unknown sample must always be performed at the same temperature at which the calibration curve was made. It is suggested that several curves be prepared at different temperatures if the laboratory does not have a satisfactory way of controlling the temperature of the sample.

4. The committee was not able to verify the precision on the order of 0.20 p.p.m. fluoride. It is, of course, frequently difficult to specify exactly the order of precision and accuracy because of the factors of concentration and interferences. In the determinations tried with distilled water containing small amounts of fluoride, deviations greater than the 0.02 p.p.m. fluoride reported by the authors were noted.

Deviations occurring in actual water samples should be even greater because of interfering ions and color.

5. Studies made by members of this committee bear out the authors' opinion concerning the advantage of the use of sulfuric acid alone for the visual procedure instead of the mixed acids used in the photometric procedure.

6. The visual comparison procedure presented is convenient and satisfactory within the limitation of the zirconium-alizarin method, but it does not offer anything new. What is offered is a concentration which does promote darker shades of color but the sensitivity or range is not increased. The authors imply an improvement in the sensitivity of the visual procedure, but it is not apparent.

CONCLUSION

The work of Megregian and Maier presents a fundamental contribution to the problem of determining fluorides in water. The committee feels that it is not the final answer but it is a definite step in the right direction. For the present and immediate future, the committee finds the Megregian-Maier modification the best on the scientific horizon for photometric purposes. Although far from ideal, it answers an imperative need. It is not felt, however, that the visual comparison procedure as proposed presents any new and distinct advantages over the present Scott-Sanchis modification which would warrant the latter's discontinuance in favor of the Megregian and Maier modification. The photometric procedure presents, for those interested, a method which generally will give good results if the proper care and precautions are exercised.

CRITICAL FACTORS IN FLUORIDE DISTILLATION TECHNIQUE

By Stephen Megregian and Ira Solet

The widespread adoption of water fluoridation has emphasized the need for rapid and accurate fluoride analysis. Although most potable waters can be analyzed for fluoride by colorimetric methods directly, interfering substances occasionally make this procedure inaccurate. The fluoride ion is quantitatively separated from most interferences by the Willard and Winter distillation technique. This method has withstood the test of time, and its various modifications are based on the needs of laboratories concerned with fluoride analysis. The present investigation was undertaken to determine those conditions which are critical and those which are superfluous to the rapid and quantitative recovery of fluoride from water samples.

The technique of fluoride distillation has been little changed since Willard and Winter (*1*) reported it in 1933. Dahle and Wickman (*2*) studied the procedure with respect to size of distilling flask, amount of nonvolatile acid used, amount of distillate collected, temperature of distillation, and presence of interferences. They concluded that a minimum of flask volume and acid volume, coupled with the highest practical distillation temperature (145° C with sulfuric acid), provided the best fluoride recovery. They also indicated that 90 percent of the fluoride was recovered in the first 50 ml. of distillate but that 100 ml. was necessary to approach 100 percent recovery. They showed that aluminum ion concentration is critical when more than 80 mg. of Al^{+++} is present in the sample.

Gilkey and others (*3*) described a modified distillation apparatus containing tetrachloroethane in an outer jacket to control the distillation temperature at 146° C. They substituted steam in place of distilled water added through a capillary, claiming that this change reduced bumping during the distillation. Thrun (*4*) distilled water samples in approximately 15 minutes by passing compressed air rapidly through a distillation apparatus heated by a large flame, with conditions so adjusted that no temperature control was necessary during distillation. He reported no appreciable blank or significant loss of fluoride in the concentrations encountered in potable waters.

The distillation technique recommended in the 1946 edition of *Standard Methods* (*5*) will produce quantitative results, but it requires approximately 2 hours for the concentration and complete separation of fluoride from the water sample, with constant attendance during that time.

EXPERIMENTAL APPARATUS

Three types of distillation apparatus were investigated. These were chosen as representing typical setups that could be assembled in most water analysis lab-

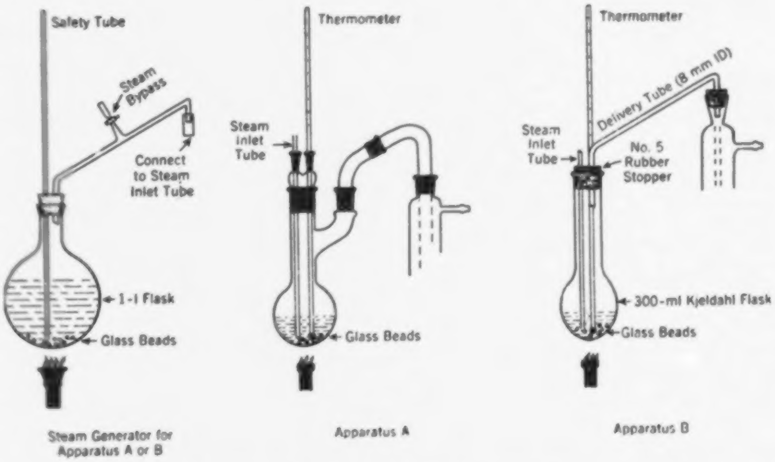

FIGURE 1.—*Fluoride distillation apparatus*

Apparatus A was purchased complete (except for a short extension to the steam inlet tube), while apparatus B was made up from common laboratory glassware.

oratories. Apparatus A (fig. 1) was purchased complete, except for a short extension that had to be welded on the steam inlet tube in order to submerge the tip in the acid. Apparatus B (fig. 1) was made up from common laboratory glassware. It was felt that the use of a 300-ml. short-neck Kjeldahl flask in place of a regular distilling flask is advantageous because the longer neck reduces contamination of the distillate by acid spray.

The diameter and shape of the discharge tube from the Kjeldahl flask is critical. Vapors and acid spray condensing in small-diameter tubing will form momentary liquid seals across the tube, and these will be carried into the distillate by the steam passing through, causing high blanks. Therefore, this tube should have a minimum inside diameter of 8 mm. and the tip protruding into the flask should be cut at an angle, thus permitting drops of spray to return to the flask. The tube should be at least 6 inches long and should connect to the condenser at an angle of approximately 30 degrees above horizontal. In the third type of apparatus, the steam inlet tube was replaced by a separatory funnel, the stem of which was drawn out to a capillary and extended below the surface of the liquid in the flask. This setup was quickly abandoned because it was difficult to operate it at 5 ml. per minute distillation rates without obtaining high blanks.

Sulfuric acid, diluted 1:1 with distilled water, was used in all of the experiments described. The fluorides were determined by the Megregian and Maier (*6*) photometric technique, using a Beckman Model B Spectrophotometer and 5.00-cm. cuvettes for most of the analyses.

Blanks

Approximately 30 ml. of 1:1 H_2SO_4 was added to 50 ml. of fluoride-free distilled water in the distilling flask and mixed well. The apparatus was assembled and the distillation carried out as will be described. Fifty-milliliter portions of distillate were collected and analyzed for fluoride. The temperature of the distillation was held between 140° C and 155° C, except for the first 50 ml., and the rate of distillation was varied up to the point at which the condensate emerged hot from the condenser. The recoveries are shown in table 1.

TABLE 1.—*Distillation of fluoride blanks*

50-ml Samples	Distillation Rate *ml/min*	F Recovered as Blank	
		mg	*ppm*
1st	8.3	−0.0002	−0.004
2nd	8.7	−0.0011	−0.022
3rd	12.5*	−0.0003	−0.006
4th	12.5*	+0.0005	+0.010
5th	13.3*	−0.0019	−0.038
6th	8.7	+0.0005	+0.010
7th	8.3	+0.0008	+0.016
8th	3.4	−0.0002	−0.004

* Condensate hot.

The standard deviation, *s*, of the Megregian and Maier (*6*) method, as determined on a series of 15 fluoride-free distilled water samples is ± 0.0008 mg. All but one of the recoveries listed in table 1 fall within the $2s$ value of 0.0016 mg., the statistical value below which 95 percent of the samples are expected to fall. Thus, the variations are no greater than those encountered by the analytical method employed, and it is safe to distill samples up to a rate of 10.0 ml. per minute without appreciable sulfate error.

Temperature of Distillation

Figure 2 is composited from four replicate runs at each of the indicated temperatures and shows the cumulative recovery of 0.050 mg. fluoride in 50 ml. distilled water added to 30 ml. 1:1 H_2SO_4; the distillate was collected and analyzed in 50-ml. portions. These curves indicate that quantitative recovery is possible within a temperature range of 130°–150° C and with a total distillate volume of 150 ml.

Volume of Distillate

In order to determine the minimum distillate volume necessary to recover fluoride quantitatively, increasingly quantities of fluoride in 50 ml. of distilled water were added to 30 ml. of 1:1 H_2SO_4 and distilled at a temperature of 140° C ± 5° C. The distillates were collected in 50-ml. portions and analyzed as before. Figure 3 illustrates the results of this series of experiments. It is apparent from these curves that 150 ml. of distillate is necessary for complete recovery within the range of fluoride quantities taken. Because, under the conditions of distillation, the first 50-ml. portions are collected as the temperature of the samples is approaching 140° C, it is necessary that at least 100 ml. of distillate be collected at the optimum temperature to insure quantitative recovery.

Rate of Distillation

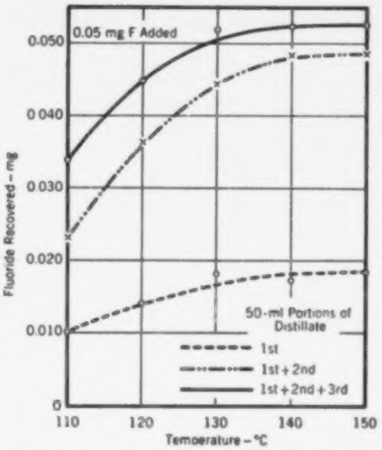

FIGURE 2.—*Temperature of distillation*

Quantitative recovery is shown to be possible in the temperature range 130°–150° C, with a total distillate volume of 150 ml. The distillation rate was 5–10 ml. per minute.

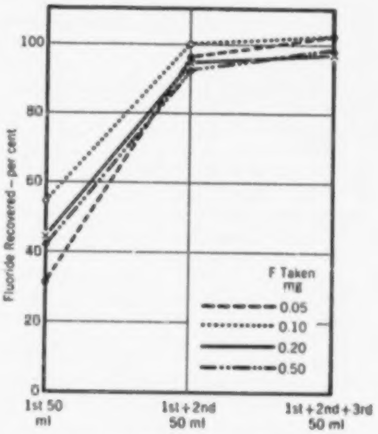

FIGURE 3.—*Volume of distillate*

The sample volume was 50 ml. and the distillation rate 5–10 ml. per minute at a temperature of 140° C ± 5° C.

Quantitative recovery of fluoride was obtained at distillation rates up to 10.0 ml. per minute with the apparatus mentioned and using the procedure to be described. Rates above this value could not be conveniently maintained because the pressure that developed in the steam generator occasionally blew out water through the safety tube, extinguishing the burner underneath and interrupting the continuity of the distillation. Quantitative recovery was also obtained at rates as low as 1.5 ml. per minute.

Interferences

Dahle and Wickman (*2*) and Willard and Winter (*1*) have determined the amounts of the various ions which affect fluoride recovery. Their work indicates that 80 mg. of Al^{+++} or 100 mg. of SiO_2 will prevent complete recovery. Other ions, such as Fe^{+++} and PO_4^{---}, which are common interferences in the subsequent determination do not affect quantitative recovery. Chloride is eliminated by precipitation as the silver salt.

The effects of aluminum and chloride were reinvestigated. Up to 20 mg. of Al^{+++} ion in 50 ml. water containing 0.1 mg. F^- did not prevent quantitative recovery when 150 ml. of distillate was collected in accordance with recommended procedure.

To study the effect of chloride, measured amounts of it were added to 50-ml. samples containing 0.1 mg. F^-, and 150 ml. of distillate was collected and analyzed as before. The results are tabulated in table 2.

The data presented emphasize the need for precipitation of chloride with silver sulfate during distillation. The effect noted above on the zirconium-alizarin reagent for fluoride is, however, in a direction opposite to that produced by chloride ions when added as NaCl to unbuffered distilled water. Since the chloride ions added in the test distill over as HCl, the effect noted must be due to the H^+ ions accompanying the chloride. As this effect will occur with all the zirconium-alizarin reagents currently in use by water analysis laboratories, it is imperative that chlorides in excess of 5 mg. be precipitated.

One other potential source of interference was investigated. It is expected that, if a significant quantity of organic matter is present in water samples, some of it may volatilize sufficiently during distillation to cause subsequent interference in the fluoride determination. Therefore, the analyst responsible for accurate fluoride analysis of unknown waters should observe the contents of the distilling flask for evidence of charring or excessive foaming and for unusual odors in the distillate, all of which are signs of possible organic interference with the subsequent fluoride determination. For example, interference was caused by one of the common wetting agents, sodium alkyl aryl sulfonate, marketed under the trade name "Nacconol NR," by Allied Chemical and Dye Corp., N.Y. Five milligrams of the detergent was added to 50 ml. of water containing 0.10 mg. of fluoride, and the sample was distilled by the recommended procedure. The analysis showed a loss of 0.025 mg. F^-. Such concentrations may be extremely rare in water; however, when evidence of organic matter is present, the analyst must resort to complete alkaline evaporation and ashing of the residue before attempting to separate the fluoride from interferences.

Procedure for Distillation

The precision of the method of fluoride analysis to be used by the analyst and the accuracy required of the final result should be the basis for selecting the volume of sample necessary for distillation.

The standard deviation, *s*, of the Megregian and Maier photometric method has been given as ± 0.0008 mg. in a 50-ml. sample. If 150 ml. of distillate are collected from the 50-ml. sample, the resultant precision of the combined distillation and fluoride determination becomes ± 0.0024 mg. or ± 0.05 p.p.m. Consequently, a 50-ml. sample containing 1.0 p.p.m. F^- can be determined, without concentration, with a resultant accuracy of ± 0.05 p.p.m. If greater accuracy is required or if the original sample is very low in fluoride content, it is recommended that a larger sample volume be taken and concentrated prior to distillation. If a sample size greater than 50 ml. is necessary, it should be evaporated down to approximately 30 ml. under alkaline conditions and transferred to the distilling flask with careful policing. The transfer is completed by washing the sides of the container with 5–10 ml. portions of fluoride-free distilled water. The total volume of sample plus wash water should not exceed 50 ml.

TABLE 2.—*Chloride interference*

Cl Added*		F Recovered mg	Cl Effect as F mg
mg	*ppm*		
10	200	0.1021	0.0021
25	500	0.1034	0.0034
50	1,000	0.1112	0.0112
100	2,000	0.1224	0.0224
300	6,000	0.1439	0.0439

* To 50 ml H_2O containing 0.100 mg F.

After the sample has been added, 8–10 glass beads, 30–40 ml. of 1:1 H_2SO_4, and sufficient solid silver sulfate are added to react with the chloride present. The contents are mixed well and the flask is closed with the stopper containing the steam inlet tube, thermometer, and outlet tube. The outlet tube is attached to the condenser and a receiver is placed under the condenser to collect the condensate. Heat is applied to the distilling flask and the steam generator. The flame under the flask is regulated to permit rapid distillation but should be confined to the surface of the flask in contact with liquid. When the temperature reaches 130° C, steam is introduced into the flask by closing the bypass, and the temperature is held at 140° C ± 5° C during the course of the distillation. At least 100 ml. of distillate should be collected with the temperature at the optimum range. This is readily accomplished under the conditions described, but, if the analyst desires to distill a sample larger than 50 ml. without prior evaporation, he should compensate for the increased sample volume by collecting an equivalent volume of distillate in excess of 150 ml. A normal distillation under the above conditions can be completed in 20–30 minutes. Distillation rates up to 10 ml. per minute can be easily maintained in the temperature range specified. The sulfuric acid residue can be reused for subsequent distillations if the accumulation of interfering ions does not exceed the limits noted. It is recommended that a blank run be made on all new setups before a distillation is attempted.

Other Considerations

The use of perchloric acid in the distilling flask in place of sulfuric acid should be avoided unless the analyst intends to complete the analysis by one of the thorium titrations methods. Also, the presence of organic matter in unknown water samples is a potential hazard when perchloric acid is used, as it has been known to explode violently under such conditions. When using sulfuric acid, the quantity of sulfate that distills over at optimum temperature is too small to interfere with the usual zirconium-alizarin colorimetric methods.

It is evident that the physical conditions necessary for rapid and quantitative distillation of fluoride are quite broad. The optimum temperature range can be easily maintained, a minimum distillate volume associated with complete recovery has been established, and the rate of distillation has been shown to have a wide range. The amounts of aluminum or gelatinous silica that would retard the distillation are far greater than those normally encountered in potable waters, and chloride can readily be precipitated. Some waters may, however, contain a significant quantity of other volatile anions such as NO_2^- and NO_3^-, which would contribute acidity to the distillate. Two hundred parts per million acidity as $CaCO_3$ in the distillate aliquot is equivalent to 0.10 p.p.m. fluoride. If this quantity is suspected, an aliquot of the distillate can be titrated for acidity with 0.1*N* NaOH and the distillate neutralized with 1.0*N* NaOH prior to the addition of fluoride reagents.

It is not necessary to protect the sides of the flask with asbestos as recommended in *Standard Methods*, provided reasonable care is exercised to prevent the open flame from continually coming into contact with the sides of the flask above the level of the liquid within. The rate of distillation of the first 50 ml. is much faster without the asbestos.

One very important source of error in performing these distillations is the presence of leaks at the various joints and connections of the apparatus. Several experiments had to be repeated because as much as 10 percent of the fluoride was lost through unnoticed loose connections.

SUMMARY

It has been shown that fluoride can be distilled quantitatively from water samples, using common, inexpensive laboratory apparatus. The optimum temperature range for distillation has been determined to be 130°–150° C. Distillation rates between 5.0 and 10.0 ml. per minute are practical, and it is necessary to collect at least 100 ml. of the distillate at the optimum temperature for quantitative recovery. Interferences that may be present in water supplies were investigated and evaluated. The apparatus requirements and a recommended procedure have been presented.

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RAPID SPECTROPHOTOMETRIC DETERMINATION OF FLUORIDE WITH ZIRCONIUM–ERIOCHROME CYANINE R LAKE

Stephen Megregian

ABSTRACT

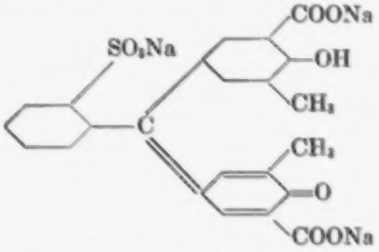
A simple, rapid technique which can be applied directly to water samples or to fluoride distillates usually gives results within 5 minutes with an accuracy within 0.02 mg. per liter of fluoride in the fluoride concentration range of 0.00 to 1.40 mg. per liter. It can replace the thorium titration for distillates in which the recognition of the thorium-alizarin end point is often very difficult. With the exception of sulfate, interference due to ions normally present in water is largely eliminated. A simple procedure for the correction of sulfate interference is included.

Biological samples, which are analyzed for fluoride content, are usually ashed, and the ash is subjected to the Willard and Winter (*11*) distillation in order to remove the fluoride from interfering substances. The fluoride in the distillate is usually determined by thorium titration. This requires concentration of the distillate, careful buffering, and a tedious titration in which the thorium-alizarin end point must be approached with great care.

Water samples are usually analyzed directly for fluoride by one of many colorimetric methods, all of which are based on the complex-forming property of the fluoride ion with various cations. These colorimetric methods are all subject, in varying degree, to the many interferences normally present in water and lack either rapidity or accuracy, or both, when applied directly to water samples.

The method proposed, when applied to distillates, eliminates the necessity for further manipulation of the distillate once it has been collectel. The fluoride value of the distillate can be obtained with great precision and accuracy within 5 minutes following the completion of the distillation. If used directly on a water sample, only the sulfate concentration need be known and a correction applied. The total time required to obtain both fluoride and sulfate readings can usually be limited to 10 minutes.

Eriochrome Cyanine R is listed as No. 722 in "Colour Index" (*5*) and is the sodium salt of *o*-sulfohydroxydimethylfuchson dicarboxylic acid. Its structural formula is given as:



Thrun (*9*) developed a colorimetric method for fluoride using the aluminum lake of Eriochrome Cyanine R at controlled pH. Richter (*4*) applied the aluminum lake to photometric measurement. Saylor and Larkin (*6*) used it as an internal indicator in the titration of fluoride with aluminum. Milton et al. (*2*), used a closely related dye, Chromazurol-S, *C.I. No. 723*, as an indicator in the titration of fluoride with thorium. Revinson and Hartley (*3*) worked out a photometric method for fluoride using thorium and Chromazurol-S, and Willard and Horton (*10*) compared many indicators for use in the titration of fluoride with thorium. They judged Eriochrome Cyanine R and Chromazurol-S to be inferior to Purpurin sulfonate and Alizarin Red S for this purpose. A search of the literature revealed no previous record of a reaction involving zirconium with Eriochrome Cyanine R.

PRELIMINARY STUDIES

The Megregian and Maier (*1*) photometric reagent [illegible] determination of fluoride ion concentration was studied to determine the optimum conditions under which this reaction should be carried out. The results of this study indicated that the reaction between zirconium, alizarin sulfonate, and fluoride ions could best be carried out in the region of 0.9*N* hydrochloric acid. At this acidity, the reaction rate between zirconium and alizarin was increased, and the fluoride effect on the reaction was more pronounced. Also, the interferences of alkalinity, chloride, and ferric iron were largely eliminated, and the degree of sulfate interference was decreased. On the other hand, the reaction became very sensitive to slight temperature variation, and low concentrations (less than 1.0 p.p.m.) of phosphate increased the reaction rate between zirconium and alizarin. When both phosphate and sulfate were present, the interference due to a given concentration of sulfate was increased.

This study indicated that it was possible to prepare a reagent which was more sensitive to slight differences in fluoride concentration, less subject to interferences, and required less time for reaction equilibrium to be reached. However, the advantages gained with this reagent were outweighed by the uncertainty imposed by the possible presence of trace quantities of phosphates in water samples and the necessity for close temperature control.

Using the knowledge gained from this study, a search was initiated for a compound other than alizarin which might undergo a color reaction with zirconium and fluoride ions without introducing errors due to minor constituents normally present in water. Over 200 dyes and other organic compounds were screened. Table I lists the compounds which were found to produce a color with zirconium and indicates the influence of fluoride on the color produced.

Table I.—*Compounds producing color change with zirconium*

Compound, (1 Ml. 0.5% per 50 Ml. Soln.)	Color in 0.1*N* HCl	Color in 0.1*N* HCl + 9 Mg. Zr/L.	Effect of Fluoride, 5 Mg./L.	Remarks
Alizarin Blue S[a]	Red	Brown	Marked	Zr lake precipitates
Alizarin Sapphire BLN[a]	Blue	Lighter blue	Weak	Blue intensifies in presence of F ions
Alizarol Azurine ECA[a]	Orange	Red	Marked	Ppt.
Benzopurpurin 4B	Blue-violet	Red	Weak	Ppt.
Carmine	Pink	Lavender	Marked	Slow reaction, ppt.
Chloranilic acid	Pink	Purple	Very weak	....
Chromazurol-S[b]	Orange	Lavender	Marked	Rapid reaction, ppt.
Curcumin	Yellow	Orange	Weak	Slow reaction
Eriochrome Cyanine R[b]	Orange	Lavender	Marked	Rapid reaction, ppt.
Quinalizarin	Orange	Violet	Marked	Slow reaction, ppt.
Quinizarin-2-sulfonate	Orange	Red	Marked	Rapid reaction, ppt.

[a] General Aniline & Film Corp., 435 Hudson St., New York, N. Y.
[b] Geigy Co., Inc., 89 Barclay St., New York, N. Y.

The choice was narrowed down to Eriochrome Cyanine R and Chromazurol-S. The former was selected for further study because: the zirconium lake is soluble and stable, equilibrium between zirconium, Eriochrome Cyanine R and fluoride ions is reached very quickly at room temperatures, the dye, as purchased, can be used immediately without further treatment, and the change in absorbance per unit fluoride per centimeter of light path is very large.

Reagents.—All of the colorimetric methods proposed for the measurement of fluoride ion concentration have a common defect—namely, the fluoride ion is not directly involved in the primary color-producing reaction. In every method proposed to date, fluoride ion concentration is measured by the amount of decolorization of the product of two reactants, one of which can form a complex with fluoride ions and can thus be restrained from entering into the color-forming reaction. A satisfactory reagent for this type of reaction should have:

A. Maximum sensitivity to fluoride ions in the desired concentration range

B. A color range which can be adequately measured by available colorimetric or photometric instruments

C. Nonsensitivity to other ions or substances commonly occurring with fluoride in the test medium

D. Conformity to Beer's law

E. A low temperature coefficient

F. High buffer capacity at the optimum pH of the reaction

G. Reagent stability

H. Reaction stability at equilibrium

I. A minimum time interval for the reaction to reach equilibrium

J. A simple procedure

None of the published methods in current use conform to all of the above ideals. The proposed method has advantages A, B, C (except for sulfate), D, E, F, G, H, I and J.

Apparatus.—Beckman Model B spectrophotometer or equivalent and 10-mm. matched cuvettes.

Preparation of the Reagents.—Reagent A. Eriochrome Cyanine R (1.800 grams) is dissolved in distilled water and diluted to 1 liter.

Reagent B. Zirconyl chloride octahydrate (0.265 gram) or zirconyl nitrate dihydrate (0.220 gram) is dissolved in about 50 ml. of distilled water. Seven hundred milliliters of concentrated hydrochloric acid (reagent grade, specific gravity 1.19) are added to the zirconium solution, and the mixture is diluted to 1 liter with distilled water.

Reference Solution.—Ten milliliters of Reagent A are added to 100 ml. of distilled water, and then 10.0 ml. of a solution prepared by diluting 7.0 ml. of concentrated hydrochloric acid to 10.0 ml. with distilled water are added. This solution is used for setting the reference point (zero) of the photometer.

Reagents A and B cannot be combined as a single reagent, because prior mixing produces precipitation on prolonged storage.

Procedure for Willard and Winter Distillates.—A 50.0-ml. portion of distillate, or aliquot diluted to 50.0 ml., and containing no more than 0.070 mg. of fluoride (1.40 p.p.m.) is taken and adjusted to a standard temperature (±2° C.). Five milliliters of Reagent A followed by 5.0 ml. of Reagent B are added to the sample and mixed well. The photometer is set at zero absorbance at 527.5 mμ (range 525 to 530 mμ.) with the reference solution and the absorbance of the sample recorded.

The fluoride value of the sample aliquot is read from a curve prepared by subjecting standard solutions of fluoride to the procedure and plotting the absorbance values at the standard temperature. Standards should be selected in the range of 0.00 to 0.070 mg. of fluoride (0.00 to 1.40 p.p.m.). The resultant curve should be a straight line of negative slope. As stated previously, the color reaction is immediate and stable. There is no waiting period for color to develop, and readings can be taken immediately, or at any other desired time without significant change in absorbance, provided the temperature is within the limits set forth. A new curve must be prepared for each fresh batch of Reagent A or B.

Procedure for Water Samples.—A 50.0-ml. water sample, or aliquot diluted to 50.0 ml. and containing no more than 0.070 mg. of fluoride (1.40 p.p.m.), is adjusted to the temperature of the standard curve (±2° C.). If the sample contains free chlorine, two drops of 0.1*N* arsenite solution are added for each part per million of chlorine present. Five milliliters of Reagent A followed by 5.0 ml. of Reagent B are added to the sample and mixed well.

The photometer is set at zero absorbance at 527.5 mμ. (range 525 to 530 mμ.) with the reference solution and the absorbance reading of the sample is taken within 5 minutes after the reagents have been added. If the absorbance reading of the sample falls beyond the range of the standard curve, the combined sulfate and fluoride concentrations of the sample are too high. In this case the procedure must be repeated with a smaller sample aliquot.

If the presence of aluminum ion is suspected in the water sample, the reaction is permitted to continue an additional 15 minutes and another reading taken. An appreciable drop in absorbance indicates the presence of aluminum ions as an interference. At this point, holding the reacted sample for 2 hours before making the absorbance reading will eliminate the interference effect of up to 5.0 p.p.m. of aluminum. Or the water sample can be subjected to the Willard and Winter distillation and the distillate analyzed according to the procedure for distillates given.

The correction for sulfate concentration in the sample aliquot is made as follows: The fluoride value of the absorbance reading is obtained from the standard curve; this value is applied to figure 3 and the sulfate error determined; the sulfate error is then subtracted from the first value to obtain the corrected fluoride value of the aliquot; and this value is multiplied by the aliquot factor to obtain the fluoride concentration of the original sample.

Absorption Spectrum.—The absorption spectra and a standard curve of the zirconium-Eriochrome Cyanine R reaction are presented in figure 1. The spectral curve for the unreacted Eriochrome Cyanine R (reference solution) is very near that of the zirconium lake. Therefore, this color system requires the incident light to be of high purity at the wave length of measurement. If a filter photometer is used for absorbance measurement, the filter should have a band pass no greater than 20 mμ. at 527.5 mμ. The wave length of 527.5 mμ. was chosen, because, as illustrated in figure 1, the standard curve measured at that value is a straight line in the range of 0.00 to 1.20 p.p.m. of fluoride with only a slight deviation from 1.20 to 1.40 p.p.m. Also, the maximum differential exists at this wave length.

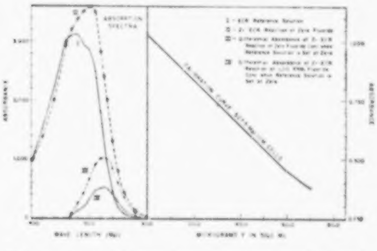


FIGURE 1.—*Absorption spectra and standard curve for zirconium-eriochrome cyanine R reaction.*

Temperature Effect.—The best temperature at which the reaction should be carried out is in the range of 22° to 28° C. Within this range a 3° C differential between the temperature of the standard curve and that of a sample is equivalent to 0.01 p.p.m. of fluoride. Thus, readings which are made at temperatures ±2° C. from that of the standard curve are well within the standard deviation of the method.

Interferences.—The Willard and Winter distillates of ashed biological samples can be analyzed directly by this procedure. The complete analysis of a distillate need not take longer than 5 minutes. The distillate need not be neutralized or concentrated, and buffer must not be added. The only possible interference would be the presence of large quantities of volatile anions, such as nitrate or unprecipitated chloride, which would come over during the distillation and produce high results in the subsequent analysis. If this is suspected, a portion of the distillate can be neutralized to the phenolphthalein end point with 1.0*N* sodium hydroxide and the neutralized aliquot taken for the fluoride analysis. The effect of large quantities of volatile anions on the zirconium-Eriochrome Cyanine R fluoride reaction is to increase the acidity at which the reaction takes place, depressing the total color produced.

For water samples which are subjected to direct analysis, the tolerance to various interferences is shown in table II.

TABLE II.—*Interferences normally present in potable waters*

Interfering Substance	Concentration, Mg./Liter, Necessary to Produce Fluoride Error Equivalent to 0.02 mg./liter F	0.10 mg./liter F
Alkalinity (as $CaCO_3$)	3300.0	Undetermined
Cl^-	3500.0	Undetermined
Fe^{+++}	10.0	40.0
PO_4^{---}	2.5	6.0
$(NaPO_3)_6$	1.0	2.0
Al^{+++}	5.0[a]	...
Free chlorine	Reduce with arsenite	
Turbidity, color	Compensate	

[a] When reaction is permitted to continue for 2 hours.

No interference was noted with calcium ion concentrations of 200 p.p.m. or magnesium ion concentrations of 100 p.p.m. The effect of very large concentrations of bicarbonate or hydroxide ions (alkalinity) is to reduce the acidity of the reaction, thereby increasing the total color produced by the zirconium-Eriochrome Cyanine R system and resulting in low fluoride values. Very large concentrations of chloride ion influence the reaction in the same direction. Ferric iron in high concentration produces a color reaction with Eriochrome Cyanine R, thus increasing the apparent color of the system and resulting in low fluoride values. None of the above interferences is normally present in sufficient quantity in water supplies to interfere with the fluoride determination.

Phosphate and hexametaphosphate (also other polyphosphates) act on the zirconium-Eriochrome Cyanine R reaction in a way similar to fluoride, in that they complex the zirconium and inhibit the color forming reaction. Water samples containing phosphates in excess of that listed in table II should be distilled prior to fluoride analysis.

Aluminum does not affect the primary color reaction at the acidity specified. It reacts with fluoride to form the complex, anion $(Al\ F_6)^{---}$ similar to $(Zr\ F_6)^{--}$. Therefore, if no fluoride is present, aluminum does not interfere. When both fluoride and aluminum are present in a sample which has been treated with the reagents, the fluoride is distributed between the zirconium and aluminum ions. Under the reaction conditions imposed, the zirconium slowly removes the fluoride from the aluminum complex and after a sufficient lapse of time (about 2 hours), the aluminum effect is no longer evident.

An interesting phenomenon occurs when aluminum is present in the sample as the metaaluminate ion—i.e., when hydroxide alkalinity is present in the original water sample. Under these conditions, the interference effect of a given concentration of aluminum on the zirconium-Eriochrome Cyanine R fluoride reaction is greatly reduced, and it disappears in 10 to 15 minutes. A logical explanation for this phenomenon would be that in solutions containing aluminum, fluoride, and hydroxide ions, the aluminum exists as the metaaluminate anion and does not complex with the fluoride present. When the reagent is added to this system, the fluoride, being in the ionic form, can immediately complex with the zirconium which has been added. By the time the aluminum ion has been released from the metaaluminate anion there is very little fluoride left to form the aluminum complex. Therefore, it exerts much less interference on the zirconium-Eriochrome Cyanine R fluoride reaction. Advantage can be taken of this phenomenon when the analyst has many samples containing both aluminum and fluoride. Simply by treating the water sample with a few drops of 1.0N sodium hydroxide until the solution is alkaline and adding the reagents as in the regular procedure, the waiting time for elimination of aluminum interference can be reduced from 2 hours to 15 minutes.

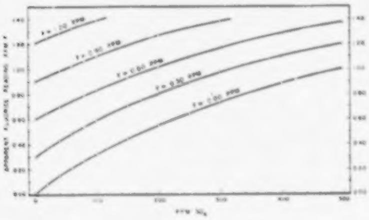


Figure 2.—*Effect of sulfate interference.*

Free chlorine and other strong oxidizing agents attack Eriochrome Cyanine R thereby reducing the total color of the system. They can be easily reduced with sodium arsenite. A slight excess of arsenite does not affect the fluoride reaction. If desired, arsenite can be incorporated in Reagent A.

Turbidity and color in the original sample can be compensated by the following technique: A 50-ml. duplicate of the water sample is taken and treated with 5.0 ml. of Reagent A. This is followed by 5.0 ml. of a hydrochloric acid solution of the same acidity as in Reagent B. The absorbance of this solution is subtracted from the absorbance of the original water sample which had been treated with both reagents. The net absorbance is then used to determine the fluoride concentration.

SULFATE ERROR AND CORRECTION

Zirconium ions in acid solution complex with sulfate ions to produce the complex $(ZrO(SO_4)_2)^{--}$ anion (*8*). Although the reaction conditions have been adjusted to reduce this effect, the zirconium sulfate complex is strong enough to prevent some of the zirconium from reacting with Eriochrome Cyanine R to produce the full color of the reaction. Therefore, the total color reduction produced in a water sample containing both sulfate and fluoride ions is the result of the sum of the two complex-forming reactions. Figure 2 shows how the presence of both fluoride and sulfate affects the fluoride reading. The data obtained in this figure were used to construct the nomograph in figure 3, which can be used to determine the sulfate error in a given sample.

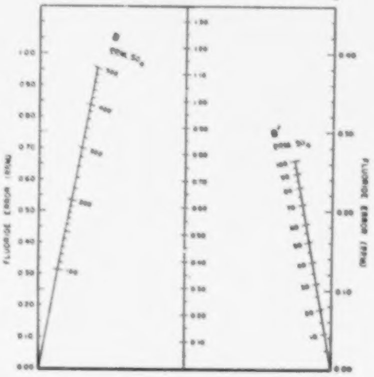


Figure 3.—*Sulfate correction nomograph.*

Figure 3 is used as follows: Obtain the fluoride reading of the sample aliquot, *A*. Determine the sulfate concentration of the aliquot, *B*. Determine the fluoride error of the sulfate concentration by running a line through *A* and *B* to *C*. Subtract the value obtained on line *C* from *A* to give the fluoride value of the aliquot. Multiply this value by the aliquot factor to obtain the fluoride content of the original sample. Lines *B′* and *C′* are expansions of *A* and *B* and should be used when the sulfate concentration is in the range of zero to 100 p.p.m.

Any procedure requiring added manipulations to remove interferences would nullify the advantages gained by using a rapid method for determining fluoride concentration. Thus, sulfate might be removed from the sample by barium precipitation or by the usual distillation procedure, but the advantages gained in using the rapid method would be counterbalanced by the time required to effect a separation by these conventional procedures. Another possibility would be to diminish the sulfate effect on the reagent by adding a known amount of sulfate to the reagent. When this is done, the reagent becomes less sensitive to fluoride, and the measurable fluoride range is reduced. Large quantities of sulfate in the reagent render it practically insensitive to fluoride, and this cannot be counterbalanced by increasing the zirconium concentration of the reagent.

Fortunately a method exists whereby the sulfate concentration of a water sample can be quickly and accurately determined, and by use of figure 3 a correction can be applied, which eliminates the necessity for time-consuming separations without materially sacrificing the accuracy of the rapid method. A slightly modified procedure based on Sheen's (*7*) turbidimetric method for sulfate is described below.

Reagents.—Barium chloride dihydrate crystals, reagent grade, 20 to 30 mesh.

Acid salt solution. Sodium chloride (240 grams) is dissolved in 900 ml. of water.

Twenty milliliters of concentrated hydrochloric acid are added and the solution is diluted to 1 liter.

Procedure.—A 50-ml. water sample or aliquot which, when diluted to 50 ml., will contain no more than 5.0 mg. of sulfate (100 p.p.m.) is transferred to a 250-ml. beaker or Erlenmeyer flask. Ten milliliters of the acid salt solution are added and mixed. Approximately 0.4 ml. (measured dry with a small spoon) of barium chloride crystals are added. Rapid swirling of the container is begun immediately and continued for 30 seconds. The sample is allowed to stand for 5 minutes, after which the resulting suspension is transferred to a photometer cuvette and the absorbance read at 525 mμ.

The sulfate concentration of the aliquot is obtained from a standard curve, which is prepared by subjecting solutions of known sulfate concentration in the range of 0.0 to 5.0 mg. (0.0 to 100 p.p.m.) to the above procedure.

ANALYTICAL DATA

Reagent Stability and Precision.—Replicate portions of four standards containing 0.00, 0.50, 1.00, and 1.40 mg. of fluoride per liter were taken on different days and treated with the same batch of zirconium and Eriochrome Cyanine R reagents. The daily absorbance readings obtained for each standard were averaged, and the individual deviations from the averages were determined in terms of fluoride concentration without regard to sign. Table III is a summary of these results. Temperatures varied between 25° and 30° C. Using the data from table III, the standard deviation throughout the range of fluoride concentrations taken was calculated to be ±0.0163 mg. per liter of fluoride.

Biological Samples.—Urine and bone samples taken in triplicate were distilled, and the fluoride content of the distillates was determined according to the following procedure: Each distillate collected was diluted to 200 ml. One hundred milliliters were taken for analysis by the thorium titration procedure,

Table III.—*Zirconium-eriochrome cyanine R reagent stability*

Reagent Age, Days	Fluoride Concentration of Standards, P.P.M. 0.00	0.50	1.00	1.40
	Deviation from Standard Curve, P.P.M.			
1 hour	0.013	0.021	0.019	0.008
1	0.006	0.002	0.013	0.027
5	0.008	0.010	0.006	0.004
9	0.008	0.004	0.015	0.020
16	0.002	0.002	0.013	0.004
42	0.008	0.023	0.019	0.004
57	0.013	0.008	0.008	0.021

Table IV.—*Biological samples*

Sample	Thorium Titration, P.P.M.	E.C.R. Method, P.P.M.
Blank	0.30	0.05
	0.28	0.04
Av.	0.29	0.045
Composite urine	0.97	0.91
	0.80	0.91
	1.03	0.95
Av.	0.93	0.92
Composite urine, av. 0.25 p.p.m. F⁻ added	1.10	1.13
	1.18	1.13
	1.23	1.13
Av.	1.17	1.13
Composite urine, 0.50 p.p.m. F⁻ added	1.45	1.41
	1.39	1.36
	1.36	1.38
Av.	1.40	1.38
Composite urine, ~1.00 p.p.m. F⁻ added	1.70	1.83
	1.81	1.81
	1.91	1.83
Av.	1.81	1.82
Bone (as % F⁻)	0.086	0.085
	0.082	0.086
	0.081	0.083
Av.	0.083	0.085

and the remaining 100 ml. were analyzed by the zirconium-Eriochrome Cyanine R method. The results for urine are expressed as the net fluoride concentration in mg. per liter after subtraction of the blank for the method used. The bone results are expressed as per cent of dry bone. The blank obtained in the zirconium-Eriochrome Cyanine R procedure includes the calcium oxide used in ashing the samples and the perchloric acid and silver perchlorate used in the distillation; the blank obtained by the thorium procedure includes, in addition, that contributed by the buffer and the titration blank. A comparison of the two methods is shown in table IV.

Examination of the individual results discloses the greater precision of the zirconium-Eriochrome Cyanine R method, and the close agreement between the two methods used. These results should not be interpreted in terms of fluoride recovery, since the distillation procedure is not a subject of this study.

Water Samples.—In order to test the applicability of the direct method to water samples, the following waters were analyzed in duplicate by distillation and by the zirconium-Eriochrome Cyanine R direct method. The Britton, S. Dak., and Bartlett, Tex., samples were analyzed as submitted. The synthetic waters A and B were prepared in the laboratory from distilled water and standard solutions of the various ions. The mineral analysis of these waters is shown below in parts per million.

	Britton, S. Dak.	Bartlett, Tex.	Synthetic A	Synthetic B
Total solids	2550	1560	...	...
Total alkalinity	260	390	32	32
Total hardness	100	180	75	75
SO_4^{--}	1100	480-500	60	60
Cl^-	330	320	56	56
Fe^{+++}	0.1	0.1-1.0	1.0	1.0
Al^{+++}	...	...	0.2	0.2
SiO_2	...	20	20	20
PO_4^{---}	...	...	3.0	3.0
F^-	6.0-6.8	7.6-8.5	0.45	1.15
Na^+	800	530	79	79

Table V is a comparison of the fluoride values obtained by the two methods. The sulfate correction used in the direct procedure was determined by applying the sulfate concentration of the sample aliquot, obtained by the turbidimetric procedure, to the nomogram in figure 3.

Factors Influencing the Selection of Reaction Conditions

The optimum concentrations of zirconium, Eriochrome Cyanine R, and hydrochloric acid, for use as a fluoride reagent, cannot be determined on stoichiometric considerations alone. Although the maximum color of the lake is developed at a molar ratio of 1 to 3 in 0.1*N* hydrochloric acid, the reagent is very sensitive to phosphate interference at this acidity. Also, it is weakly buffered, and slow to reach equilibrium. Increasing the acidity reduces phosphate interference and increases buffer capacity and reaction rate. However, the reaction is no longer stoichiometric, and some reduction in fluoride sensitivity occurs, along with a reduction of the total color produced by the reaction (fig. 4).

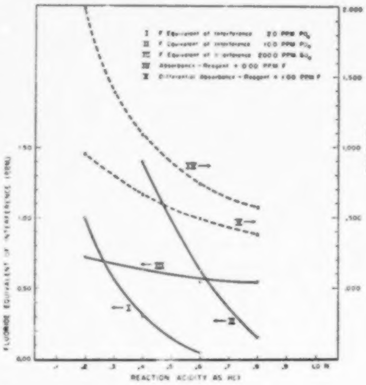


Figure 4.—*Effect of reaction acidity on zirconium-eriochrome cyanine R reagent*

Some of the color lost can be regained by increasing the Eriochrome Cyanine R concentration of the reagent above the 1 to 3 ratio. However, too great an excess cannot be tolerated, because the excess dye absorbs strongly at the wave length of measurement.

The zirconium concentration determines the amount of initial color which the reaction will produce, and the effective fluoride range. It is limited by the ability of the spectrophotometer to measure deep colors accurately.

The selection of the reaction conditions, as presented above, was based on these factors and is believed to be near

Table V.—*Water samples*

	Distillation				E.C.R., Direct[a]						
Source	Ml. sample distilled (200 ml. collected)	F^- concn. of distillate, p.p.m.	Aliquot factor	Net F^-, p.p.m.	Ml. sample (diluted to 50 ml.)	SO_4^{--} concn. of aliquot (turbidimetric), p.p.m.	Apparent F^-, p.p.m.	SO_4^{--} corr. (Figure 3), p.p.m.	F^-, in aliquot p.p.m.	Aliquot factor	Net F^-, p.p.m
Britton, S. Dak.	25	0.81	8	6.48	5	109	0.92	−0.27	0.65	10	6.50
		0.80		6.40			0.94		0.67		6.70
Av.				6.44							6.60
Bartlett, Tex.	25	1.01		8.08	5	48	0.96	−0.13	0.83	10	8.30
		1.03		8.24			0.97		0.84		8.40
Av.				8.16							8.35
Synthetic A (F^- added = 0.45 p.p.m.)	100	0.24	2	0.48	50	59	0.58	−0.18	0.40	1	0.40
		0.19		0.38			0.60		0.42		0.42
Av.				0.43							0.41
Synthetic B (F^- added = 1.15 p.p.m.)	50	0.29	4	1.16	25	30	0.69	−0.10	0.59		1.18
		0.30		1.20			0.67		0.57		1.14
Av.				1.18							1.16

[a] Corrected for sulfate.

the optimum with respect to the various considerations previously listed. When 5.0 ml. of each of the reagents are added to a 50-ml. sample, it contains 0.375 mg. of zirconium and 9.0 mg. of Eriochrome Cyanine R (molar ratio 1 to 4) and the solution is 0.7*N* in hydrochloric acid. Under these conditions 0.050 mg. of fluoride (1.00 p.p.m.) will produce a change in absorbance of 0.480 unit using a 1.0-cm. light path. The Beckman Model B spectrophotometer can usually be read with precision to 0.005 absorbance unit. Thus, it is possible to detect changes in fluoride concentration of 0.5γ of fluoride in a 50-ml. sample and to measure with precision 0.050±0.016 mg. of fluoride per liter. By scaling down proportionately the amounts of the reagents added, it is possible to determine as little as $0.25\ \gamma$ of fluoride in a 5.0-ml. sample.

Other Applications

At low acidities (up to 0.1*N*) Eriochrome Cyanine R produces a color with many cations such as tin(IV), thorium(IV), titanium(IV), iron(III), aluminum(III), and lead(II). As the acidity is increased, only zirconium(IV) and, to a lesser extent, hafnium(IV), produce a color reaction. In a reaction medium containing 0.2*N* hydrochloric acid, the absorbance change per cm of light path per p.p.m. of zirconium is about 0.400 unit when the zirconium concentration lies in the vicinity of 4.0 p.p.m. Thus, by proper control of reaction acidity, a method could be developed, using Eriochrome Cyanine R, which would determine small amounts of zirconium in the presence of many cations which usually interfere in other procedures.

SUMMARY

A rapid spectrophotometric determination of fluoride ion concentration uses the reaction between zirconyl ions and Eriochrome Cyanine R for color formation and the subsequent decolorization of the lake by fluoride ions. The reaction is immediate, stable, and follows Beer's law in the concentration range of 0.00 to 1.40 mg. of fluoride per liter. The standard deviation in this range is ±0.0163 mg. of fluoride per liter using standard photometer cells with a 10-mm. light path. Use of this method, in place of the familiar thorium titration on distillates of biological samples, will save considerable time and increases precision. When the method is applied directly to water samples, the interference caused by many ions commonly present in water is largely eliminated. Only sulfate ion concentration must be known and a correction applied.

ACKNOWLEDGMENT

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DILUTION OF FLUOSILICIC ACID

Ervin Bellack and F. J. Maier

When undiluted fluosilicic acid is fed directly into water to produce a concentration of approximately 1.0 p.p.m. fluoride, no particular difficulties are encountered. When attempts are made to use this acid at smaller plants, however, where limitations of the chemical feeders have required that the acid be diluted prior to feeding, considerable difficulties have been experienced. These difficulties have arisen when the acids are diluted in the order of more than ten parts of water to 1 of acid, resulting in the formation of a visible, insoluble precipitate which clogs the feeder and its appurtenances, valves, pumps, and orifices. The composition of this precipitate has been investigated and it was found to be finely divided silica.

The precipitate forms regardless of the nature of the water used for dilution of the acid, and distilled water causes it to form as readily as does water containing more than 2,000 p.p.m. of dissolved solids. The amout of dilution is critical, however. Beyond a ratio of about 20 to 1, when an acid containing the greatest silica content obtainable is used, no precipitate is formed. Not all of the commercially available acids exhibit this precipitate-forming characteristic, however.

METHODS OF PRODUCTION

Two methods are used for producing this acid. The more common involves the absorption by water of silicon tetrafluoride, produced by the acidification of phosphate rock. The fluosilicic acid produced by this process contains appreciable and varying amounts of free silica. The other method is essentially a controlled reaction of silica in aqueous hydrofluoric acid, and the fluosilicic acid thus formed is of higher strength and, being practically free of colloidal silica, will not form a precipitate on dilution. Because this method is more expensive, however, most of the fluosilicic acid used for water fluoridation is produced by the former method, resulting in greater amounts of free silica.

Use of Hydrofluoric Acid

To prevent the formation of objectionable precipitate, it was thought possible either to stabilize the colloidal suspension or remove the excess silica by physical or chemical means. To this end, several additives were tried, but only strong acids gave any measure of success, and of these, hydrofluoric acid seemed most promising. Hydrofluoric acid not only dissolves the colloidal silica, but is entirely compatible with the fluosilicic acid solution equilibria:

$$H_2SiF_6 = 2H^+ SiF_6^{--}$$
$$SiF_6^{--} + 2H_2O = SiO_2 + 4H^+ + 6F^-$$

With the commercial acids available, the optimum ratio for the elimination of precipitation was found to be 0.2 ml. of 48 percent hydrofluoric acid to 10.0 ml. of 30 percent fluosilicic acid. The commercial fluosilicic acid could then be fortified on the basis of 1 gallon of hydrofluoric acid to 50 gallons of fluosilicic acid. The addition of this amount of hydrofluoric acid would alter the corrosive characteristics of the mixture very little and the increased chemical and handling costs would be offset by the higher fluoride ion concentration in the resulting dilution. Optimum ratios for acids having different colloidal-silica concentrations would necessarily have to be determined by the acid manufacturers. In the interests of safety and maximum effectiveness, these manufacturers should also stabilize the acid at their plants by adding the optimum quantities of hydrofluoric acid.

ANALYTICAL APPLICATIONS OF ZIRCONIUM ELECTRODE

Stephen Megregian

The current generated by the spontaneous electrolysis of the zirconium-platinum cell is proportional to fluoride concentration between 2.5 and 20.0 mg. per liter. The determination may be performed in from 5 to 30 minutes, and the apparatus required is simple to assemble. Zirconium metal can also be used as an indicator electrode in titrations of the zirconyl ion with fluoride. This electrode exhibits a sharp rise in potential when as little as 0.25 mg. of fluoride in 100 ml. is present in solution. The titer of the fluoride solution for zirconium depends upon the acidity of the zirconyl solution. At constant acidity zirconium can be titrated with fluoride. The reverse titration is not feasible.

The procedure of Baker and Morrison (*1*) for determining fluoride ion concentration by means of the current generated by an aluminum-platinum cell was investigated to determine if it could be used in the control of fluoridation of public water supplies. Because the chloride ion which interferes with this procedure could not be removed by simple means short of the Willard-Winter (*2*) distillation the method was not considered adaptable to the routine analysis and control of fluoridated waters. However, it was believed that other electrode systems might be found which would be more specific in their response to the presence of the fluoride ion.

The metals to be considered would be among those high in the electromotive series and whose ions would unite with fluoride to produce a complex ion in acid solution. The following metals were tested for sensitivity to a fluoride ion concentration of 20 mg. per liter in approximately 0.1*M* perchloric acid solution: aluminum, beryllium, iron, germanium, niobium, lead, tantalum, titanium, and zirconium. None showed a sensitivity to fluoride ion at this concentration except zirconium. Therefore, only the zirconium-platinum electrode system was studied further.

During the course of this investigation it was discovered that the zirconium electrode exhibited a sharp rise in potential when as little as 2.5 mg. per liter of fluoride was present in solution. This observation indicated that the electrode might be a useful indicator in reactions involving the fluoride ion as titrant, and the conditions under which this titration might be carried out were also studied.

DETERMINATION OF FLUORIDE BY SPONTANEOUS ELECTROLYSIS

Materials.—The zirconium electrode was a ⅛-inch-diameter wire of the metal obtained from the Carborundum Metals Co., Inc., of Akron, N. Y. The platinum electrode was a strip of commercially available metal. The electrodes were coated with wax except for about one-half inch of the lower portions. Fluoride solutions were prepared by diluting a stock solution of 0.526*M* sodium fluoride (1 ml. equals 10 mg. of fluoride). The acids used were the standard reagent grade. The current was measured by the galvanometer circuit of a Sargent Model III Polarograph.

Procedure.—A 100 ml. sample of the fluoride solution was acidified with the appropriate acid to a predetermined concentration in a 250 ml. beaker. The beaker was placed over a magnetic stirrer set at a predetermined speed, and the electrodes were immersed in the solution. The galvanometer circuit was closed and after a time interval of 5 to 30 minutes, during which the current reached stability, the reading was taken.

Experimental.—Various concentrations of several acids were tested to discover the optimum conditions for the reaction. Either perchloric or hydrochloric acid could be used, although perchloric was preferred because the current reached stability somewhat sooner than with the hydrochloric acid. Sulfuric acid or phosphoric acid could not be used because these rendered the zirconium electrode insensitive to fluoride at the concentration levels tested. It appears that the concentration of acid is not critical and that any strength between 0.1 and 1.0*M* can be used without appreciable change in sensitivity to fluoride. A linear relationship exists between fluoride concentration and current in fluoride solutions containing 0.1*M* perchloric acid.

The maximum concentration of fluoride which may be determined by this procedure would depend on the range of the galvanometer and the surface area of the electrodes. The lowest concentration which could be measured with precision was about 2.5 mg. of fluoride per liter. Below this value the cell became insensitive to small changes in fluoride. Attempts made to sensitize the zirconium electrode to the concentrations of fluoride of interest in water fluoridation were fruitless.

The use of this technique with relatively pure fluoride solutions will probably produce satisfactory results. The following precautions must be exercised throughout a series of determinations:

Electrode area, electrode spacing, stirring rate, and temperature of the test solution must be held constant.

When the calibration curve is prepared, the highest concentration of fluoride should be run first, followed by the other standards in the order of decreasing concentration. When an unknown is run, the cell should first be sensitized by immersion in the highest concentration of

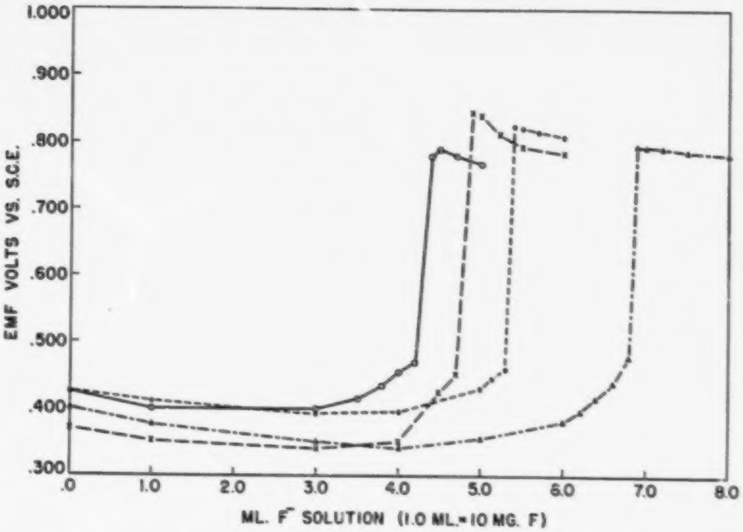

FIGURE 1.—*Titration curves at various acidities.*

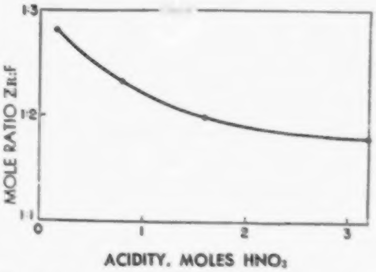

FIGURE 2.—*Effect of solution acidity on mole ratio of zirconium to fluoride end point.*

the fluoride standards until the current output agrees with that of the standard curve; then the electrodes may be transferred to the unknown solution. No further sensitization is necessary during a series of determinations so long as the fluoride concentration of the unknown does not fall below 2.5 mg. per liter.

The elapsed time between the closing of the galvanometer circuit and the stabilization of the current produced will vary from 5 to 30 minutes, with the lower fluoride concentrations requiring the longer time interval.

POTENTIOMETRIC TITRATION OF ZIRCONIUM WITH FLUORIDE

Materials.—Zirconium oxychloride, prepared by dissolving 35.4 grams of the salt in 1 liter of water. The solution contained 11.6 mg. of zirconium per liter.

Sodium fluoride, 0.526*M*.

Nitric acid, concentrated, reagent grade.

Saturated calomel reference electrode.

Zirconium wire electrode, same as before.

Beckman Model G pH meter.

Procedure.—A predetermined quantity of the standardized zirconium solution was transferred to a beaker. A predetermined volume of nitric acid was added and the solution diluted to 100 ml. The beaker was placed on a magnetic stirrer, the electrodes were immersed in the solution, and it was titrated with the standard sodium fluoride solution. As the end point was approached, each small addition of the fluoride solution tended to produce a sudden rise in potential followed by rapid oscillations up and down until a stable potential was reached slightly higher than that produced by the previous increment. However, when the end point was exceeded, a rapid rise in potential occurred which oscillated only slightly between its maximum and minimum readings. Further addition of titrant tended to decrease the potential of the electrode gradually. The end point of the titration was taken at the occurrence of the sharp rise in potential.

RESULTS

Nitric acid was preferred to the other mineral acids for this titration because the potential readings were more stable.

Figures 1 and 2 illustrate the effect of acid concentration on the stoichiometric relationship between zirconium and fluoride ions. It can be seen that

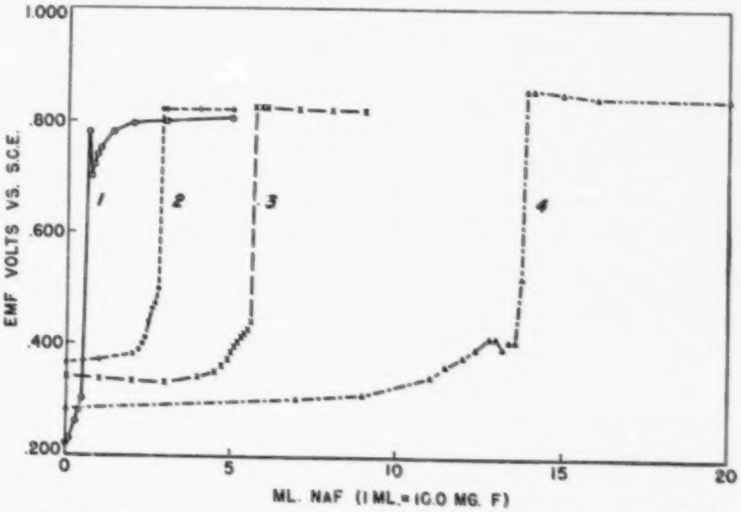

FIGURE 3.—*Titration of zirconium ion with sodium fluoride at constant acidity of 0.5M nitric acid*

Curve	Mg. Zr	Meq. Zr	Ml. NaF	Meq. F	Mole Ratio, Zr to F
1	11.6	0.127	0.65	0.34	1 to 2.70
2	58.0	0.635	2.90	1.53	1 to 2.40
3	116.0	1.270	5.70	3.00	1 to 2.35
4	290.0	3.180	13.90	7.32	1 to 2.30

acidity strongly influences the stoichiometry of this reaction. Consequently, all titrations should be carried out under conditions of constant acidity.

Figure 3 is an illustration of a series of titrations at constant acidity with various quantities of zirconium in solution. Except for the sample containing the least quantity of zirconium, the ratio of zirconium to fluoride is nearly constant under these conditions, indicating that a quantitative relationship can be developed for most ranges of zirconium concentration.

Fluoride cannot be determined directly by using a zirconium solution as titrant because a sharp potential drop is not produced when small increments of zirconium are added near the point.

DISCUSSION

It is obvious that the potential curves do not have the same shape as the typical S curves obtained in the usual potentiometric titration. This is probably due to the fact that this titration does not follow the Nernst equation, because the rise in potential occurs when excess fluoride ion is present rather than in the presence of zirconium ions, as would be necessary for the Nernst equation to hold. What probably occurs is that under the conditions existing in solution the zirconium electrode is normally covered with a film of oxide and exhibits a potential due to this film. When fluoride ion is present in the solution it attacks the oxide layer, dissolving it and exposing the true metal surface to the solution, thus exhibiting the true potential of the zirconium metal.

Possibly other titrations could be carried out using the fluoride ion as titrant in acid solutions in which the zirconium electrode can function as the indicator. It should be possible to titrate thorium, lanthanum, beryllium, aluminum, and other ions capable of producing a complex fluoride anion. Also, lead could be titrated as lead chlorofluoride. The aluminum electrode might also be used as an indicator in those titrations in which no mineral acid is present and the chloride ion is absent.

Although this research is incomplete, it is reported now because this laboratory has no further interest in its development. The data presented may be of sufficient interest to stimulate further work with these electrodes.

ACKNOWLEDGEMENTS

The author is indebted to the Aluminum Co. of America for its donation of various aluminum alloys, the Brush Beryllium Co. for its donation of beryllium foil, the Carborundum Metals Co., for its donation of zirconium wire, and the Fansteel Metallurgical Corp. for its donations of niobium and tantalum sheet, all of which were used in this study.

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CHROMATOGRAPHIC SEPARATION OF FLUORIDE AND PHOSPHATE

By ISADORE ZIPKIN, WALLACE D. ARMSTRONG, and LEON SINGER

SIR: The determination of fluoride in biological materials requires its separation from interfering ions, chief among which is phosphate. This may be accomplished by the Willard and Winter distillation (*9*) or by the diffusion technique of Singer and Armstrong (*8*).

Recent advances in ion exchange and partition chromatography suggest that these procedures may also be feasible for the separation of fluoride and phosphate, as the relative affinities of these ions for an anion exchange resin differ greatly. Funasaka, Kawane, and Kojima (*5*) have reported the separation of fluoride from phosphate (1 to 60), using Amberlite IRA 410 in the hydroxyl form.

In the present studies, as little as 25 γ of fluoride have been separated quantitatively from 500 times as much phosphorus on Dowex-1 (OH^-), using phosphorus-32 as tracer indicator. In addition, fluoride has been separated semiquantitatively from phosphate by a simple paper partition chromatographic technique.

EXPERIMENTAL AND RESULTS

Ion Exchange Chromatography. Dowex 1-X10, 100–200 mesh, chloride form was cycled three times, using 100 ml. of 3*N* sodium hydroxide and 3*N* hydrochloric acid for each cycle, and then was left in the hydroxyl form. Resin bed dimensions were 10.5×1.0 cm. One hundred and twenty drops per tube were collected at 8 to 9 drops per minute, so that approximately 6 ml. were collected. The eluates were received in tared test tubes mounted on a Packard fraction collector and the volumes were calculated. Two hundred micrograms of fluoride as sodium fluoride in 0.5 ml., 100 γ of phosphorus as potassium dihydrogen phosphate in 0.5 ml., and 1.0 ml. of a phosphorus-32 solution (Catalog No. P-32-P-1, Oak Ridge National Laboratory, Oak Ridge, Tenn.) containing approximately 25 μc. per ml. were mixed and placed on the column.

The gradient elution technique (*3*) was used to find the minimal concentration of alkali sufficient to separate fluoride quantitatively from phosphate. Gradients were established by introducing a 1*N* sodium hydroxide solution from a Mariotte-tube-controlled separatory funnel into a constant-volume mixing chamber containing 250 ml. of water. Mixing was provided by a magnetic stirrer, and the eluting fluid was transferred to the resin bed by a narrow-bore rubber tubing to minimize lag. All samples were carefully neutralized to *p*-nitrophenol end point with hydrochloric acid and made up to 10.0 ml.; and 3.0 ml. or less was taken for direct analysis for fluoride—i.e., without distillation—according to the authors' modification of the Icken-Blank procedure (*7*). Aliquots of 0.1 ml. from each tube were plated in shallow stainless steel planchets and counted, using a conventional end-window Geiger-Müller tube. When corrected for the blank, 99.3 percent of the added fluoride was recovered in tubes 26 to 29. Of the original phosphorus-32 activity 99.5 percent, corrected for background and decay, was recovered, and none was found in any of the tubes containing fluoride. These data are presented in figure 1.

On the basis of the gradient elution study, it appears that a concentration of 0.5*N* sodium hydroxide should quantitatively separate fluoride and phosphate.

The same apparatus was used, except that no gradient was employed. Samples were collected as previously described—that is, 120 drops per tube at a flow rate of 8 to 9 drops per minute. A new column was prepared, and 25 γ of fluoride in

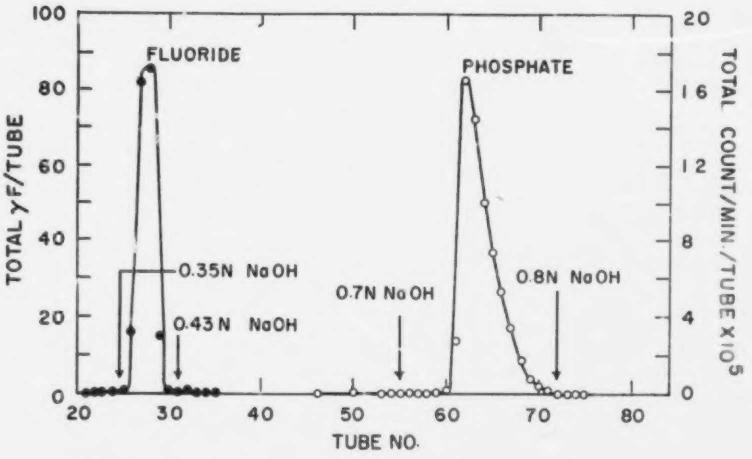

FIGURE 1.—*Gradient elution curve for fluoride and phosphate.* 200 γ of F as NaF, 100 γ of P as KH_2PO_4, and 25 μc. of P^{32} placed on column

0.5 ml. as sodium fluoride, 12.5 mg. of phosphorus in 0.5 ml. as potassium dihydrogen phosphate (F/P=1/500), and 1.0 ml. of phosphorus-32 as tracer indicator were mixed, placed on the column, and eluted with 0.5*N* sodium hydroxide as previously described. Of the fluoride, 99.2 percent, corrected for the blank, appeared in tubes 7 to 9 (10.0, 12.3, and 2.5 γ, respectively), and the first evidence of radioactivity appeared in tube 29. Hence, 25 γ of fluoride have been quantitatively separated from 500 times as much phosphorus present as phosphate.

Paper Partition Chromatography. For paper partition chromatography, the ammoniacal methanol solvent of Burrows, Grylls, and Harrison (*2*) was used (70 volumes of absolute methanol and 30 volumes of 2*N* ammonium hydroxide). Whatman No. 3MM filter paper strips (Chicago Apparatus Co., Chicago, Ill., Cat. No. 24503R) prepared according to Armstrong and Carr (*1*) were used in the simplified milk bottle technique of Gage, Douglass, and Wender (*6*).

Ten micrograms of fluoride as sodium fluoride in 0.01 ml. were separated from 20.0 γ of phosphate as potassium phosphate in 0.01 ml. Based on six runs, an R_f value of 0.65 was obtained for fluoride whether chromatographed alone or with phosphate. Under identical conditions phosphate had an R_f value of 0.34, which was unaffected by the presence of fluoride. Phosphate alone appeared as an immediate yellow spot on a colorless background when sprayed with the ammonium molybdate reagent (*4*). When fluoride and phosphate were superimposed on the same strip and chromatographed, the zirconium-alizarin spray reagent (*4*) was found applicable for both, but the phosphate spot required 1 to 2 hours for full attainment of the yellow color.

CONCLUSIONS

Twenty-five micrograms of fluoride have been quantitatively separated from 12.5 mg. of phosphorus (F/P=1/500) on Dowex-1 (OH^-) using 0.5*N* sodium hydroxide. Fluoride and phosphate may be easily separated qualitatively by paper partition chromatography on Whatman No. 3MM strips, using an ammoniacal methanol solvent. Both ions are identified by the zirconium-alizarin spray reagent.

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STUDY OF PERMANENT COLOR COMPARATORS FOR FLUORIDES—FLUORIDATION MATERIALS AND METHODS

By FRANZ J. MAIER and TASK GROUP 2620 P, AWWA

A large number of water plant laboratories are using permanent color standard kits, not only for measuring the concentration of fluorides, but also for determining many other substances ordinarily found in water. One reason for this widespread acceptance is the fact that the kits provide a previously prepared and relatively stable color standard for the concentrations ordinarily encountered. In addition, the procedures for testing the sample are generally simple and the instruments and reagents are inexpensive when compared with other laboratory methods. Many communities which have adopted controlled fluoridation, and particularly those with no water works laboratories, are using kits which are produced by either of two manufacturers. These manufacturers are the Hellige Company, Inc., Garden City, N.Y., whose kit will be referred to in this article as Kit A, and the W. A. Taylor Company, Baltimore, Md., whose kit is designated Kit B.

The manufacturers of the permanent color standard kits have specified procedures for obtaining results of an accuracy (0.10 mg/l) comparable to that indicated to be obtainable by using techniques described in *Standard Methods* (*1*). Deviations from specified procedures result in inaccuracies or, at worst, a complete inability to match colors. Possibly because of failure to observe the manufacturers' directions, many water plant operators and analysts have criticized the performance of these kits. As a result of its 1954 survey on the use of Kits A and B, Task Group 2620 P reported (*2*):

> Several states indicate that results obtained from either set will vary because of variations in strength of reagents, one state reporting deviations up to 0.7 p.p.m. Another state reports both kits are subject to temperature variations and give results as much as 0.5 p.p.m. higher than actual concentration in hot weather.

Because of these problems and reports, a detailed study of fluoride kits was undertaken to determine if the kits are accurate to 0.10 mg/l fluoride when used under operating conditions which prevail in water works laboratories. It was considered equally important to determine those factors which could produce and error in excess of 0.10 mg/l fluoride.

PROCEDURE

The AWWA furnished new instruments, slides, disks, and reagents from both kits, and these were received at all laboratories at approximately the same time. The instruments were assembled according to the manufacturers' recommendations and the reagents were prepared, divided, and stored as specified on the form shown in figure 1.

The reagent of Kit A was investigated under four different conditions of preparation and storage:

1. The single reagent was mixed at the manufacturer's plant and, when received by the various participating laboratories, was stored "refrigerated and protected from light" according to the manufacturer's directions.
2. Solutions of zirconium oxychloride, sodium alizarin monosulfonate, and acid dilutions were received, mixed according to directions, and divided into three portions. (The manufacturer provides the ingredients of the reagent so that it can be made up as required.) One portion was stored according to the manufacturer's directions, mentioned above: "refrigerated and protected from light."
3. The second portion of reagent was stored at room temperature in daylight but not in direct sunlight.
4. The third portion was stored at room temperature in the dark.

The reagent of Kit B was tested under the following conditions of preparation and storage:

1. The mixed reagent as received was, as recommended by the manufacturer, "kept in a cool place protected from direct sunlight and contamination . . . (Storage in a refrigerator may help to increase its useful life.)"
2. The mixed reagent as received was stored at room temperature in daylight but not in direct sunlight.
3. The mixed reagent as received was stored at room temperature in the dark.
4. The mixed reagent as received was stored as in Condition 1 above and used in the instrument without the illuminator—that is, the readings were made using the natural light available at a window.

In each of three successive samples on both kits the first was run undiluted, the second was diluted one-half, and the third diluted one-fourth. This was done in order to assess the extent of variation from *Standard Methods* readings as concentrations changed and as storage conditions varied.

The laboratory study started in December 1955 in most of the participating laboratories and the first part of the work ended in May 1956. A study to determine the useful life of the Kit A reagent is continuing; there is no discernible change in error at the end of 13 months.

The procedure was standardized as closely as possible for the various participating laboratories. The water samples tested were those being routinely examined. All water supplies in-

COMMITTEE ON FLUORIDATION METHODS AND MATERIALS—AWWA

PERMANENT COLOR COMPARATORS FOR FLUORIDES

Laboratory————————

Analyst————————

Scott-Sanchis or Lamar Reagent			Hellige Instrument (Recommended: 60–65 min; 23–27°C; 50 ml)										Taylor Instrument (Recommended: 1 hr; 20–30°C; 50 ml)									
Date	Reagent Condition	Fluoride—mg/l	Time (One Sample at 25°C) (±2 min)					Temperature (Five Samples) (±1°C)					Time (One Sample at 25°C) (±2 min)					Temperature (Five Samples) (±1°C)				
			30	45	60	75	90	15	20	25	30	35	30	45	60	75	90	15	20	25	30	35
	1																					
	2																					
	3																					
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Hellige Reagent

Reagent Condition

1. As received from manufacturer and stored in refrigerator at 5–10°C. (Record date received.) ————
2. Mixed from manufacturer's ingredients and stored in refrigerator at 5–10°C. (Record date mixed.) ————
3. Mixed as in No. 2, but stored at room temperatures in daylight but not in direct sunlight. (Record approximate average temperature.) ————
4. Mixed as in No. 2, but stored in dark (not refrigerated). ————

Taylor Reagent

Reagent Condition

1. Mixed by manufacturer and stored at 5–10°C in refrigerator. (Record date received.) ————
2. As in No. 1, but stored at room temperature in daylight (not in direct sunlight). (Record approximate average temperature.) ————
3. As in No. 1, but stored at room temperature in dark. ————
4. Mixed and stored as in No. 1, but read in window light. (Record dull, cloudy, or bright.) ————

FIGURE 1.—*Standard form used in AWWA tests of permanent color comparators*

volved employed controlled fluoridation, except in Los Angeles where the natural fluoride content is sufficiently high. Each sample required a volume of at least 700 ml., which was collected in one container, mixed, dechlorinated if necessary, and divided into the portions as shown on the reporting form. (Sodium arsenite was used in dechlorination as specified in *Standard Methods.*)

The dechlorinated sample was then divided into at least one 100-ml. portion and twelve 50-ml. portions. Those portions used for temperature evaluation were brought to the required temperatures. The single portion needed for the "time" reading generally was the one prepared for the 25° C temperature tests. The fluoride standards for use with the *Standard Methods* Scott-Sanchis or Lamar reagent were set up at 0.05 mg/l intervals on either side of the estimated fluoride content of the sample. The reagent used for this purpose was prepared in each laboratory and stored as specified in *Standard Methods.*

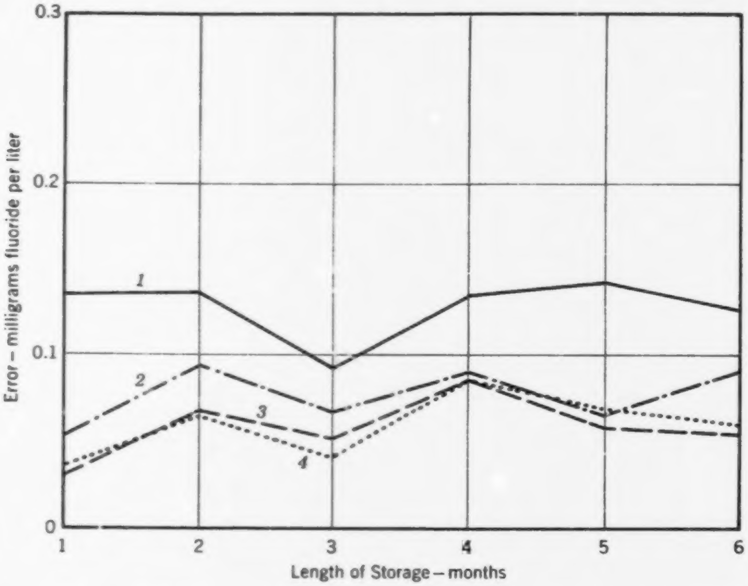

FIGURE 2.—*Effect of reagent storage on accuracy of Kit A. Numbers 1–4 represent the correspondingly numbered conditions of preparation and storage of the reagent referred to in the text.*

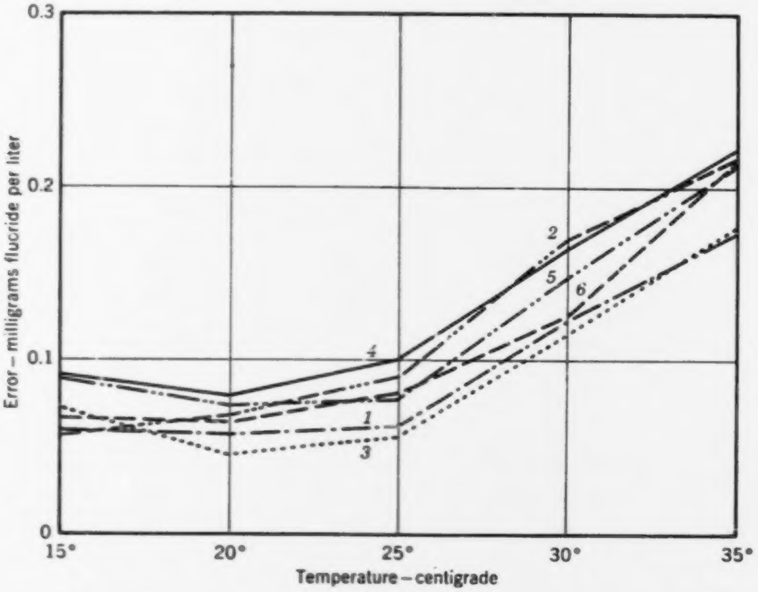

FIGURE 3.—*Effect of reaction temperature on accuracy of Kit A. Numbers 1–6 represent age of reagent (in months) at time of test.*

As shown on the form in figure 1, the samples were examined at various times (±2 minutes) and temperatures (±1° C). Water baths were used to maintain these temperatures during the reaction. The manufacturers' recommended reaction times, temperatures, and quantity of sample are also shown on the form (fig. 1).

Generally, each laboratory completed the preparation of one form each month. This represented the examination of twelve samples monthly, with ten readings made on each sample, using both Kit A and Kit B procedures. It also included at least one sample (for each of the twelve) measured according to the visual *Standard Methods* technique preferred by the laboratory.

In calculating the average error of the readings, the gross error of each analysis was computed by obtaining the difference between the *Standard Methods* result and that obtained with each kit. The data plotted (figs. 2–7) are arithmetical averages of these errors without respect to sign. With Kit B, whenever a sample produced a color representing a value of fluoride less than zero, its value was taken as zero for calculation purposes.

RESULTS—Kit A

Kit A almost always showed a positive error—that is, when the water sample contained 1.0 mg/l fluoride, it ordinarily gave a reading higher than 1.0 mg/l. Figure 2 shows that, despite the age of the reagents, the error is well within 0.10 mg/l, except when using the reagent mixed by the manufacturer. Better results are obtained with the reagent prepared by the laboratories with ingredients supplied by the manufacturer. Various storage temperatures and storage in darkness or daylight had no apparent effect on the reagent.

Figure 3 shows that the optimum reaction temperature is between 15° C and 25° C. Little difference in error can be detected by using any more precise temperature conditions. Beyond 25° C the amount of error increases rapidly. No correlation could be detected between the age of the reagent and the reaction temperature.

Figure 4 indicates that the duration of the reaction should exceed 60 minutes. There is a very slight tendency for the error to decrease as the reaction time is increased (up to 90 minutes). There does not appear to be any loss of accuracy as the reagent life is extended to at least 6 months.

Figure 5 portrays graphically the relation of error to fluoride range, as well as the effects of the various storage conditions within each range. Only a very slight difference in error can be observed between the ranges.

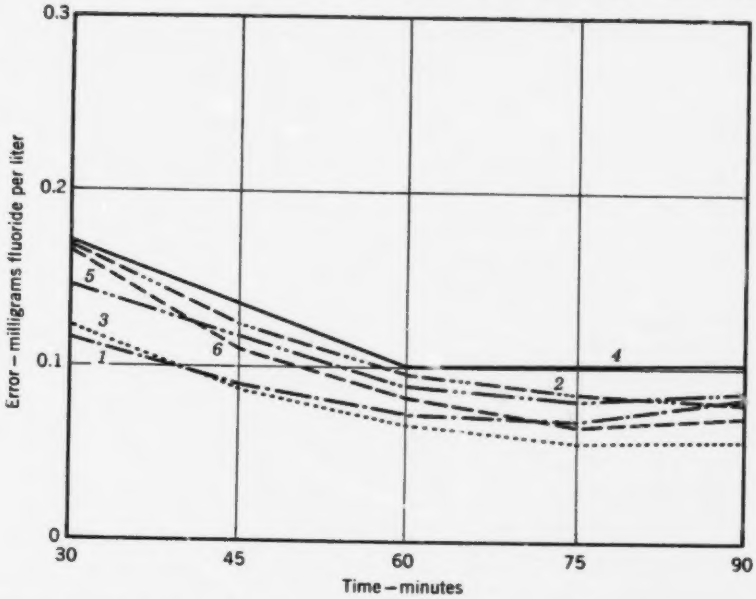

Figure 4.—*Effort of reaction time on accuracy of Kit A. Numbers 1–6 represent age of reagent (in months) at time of test.*

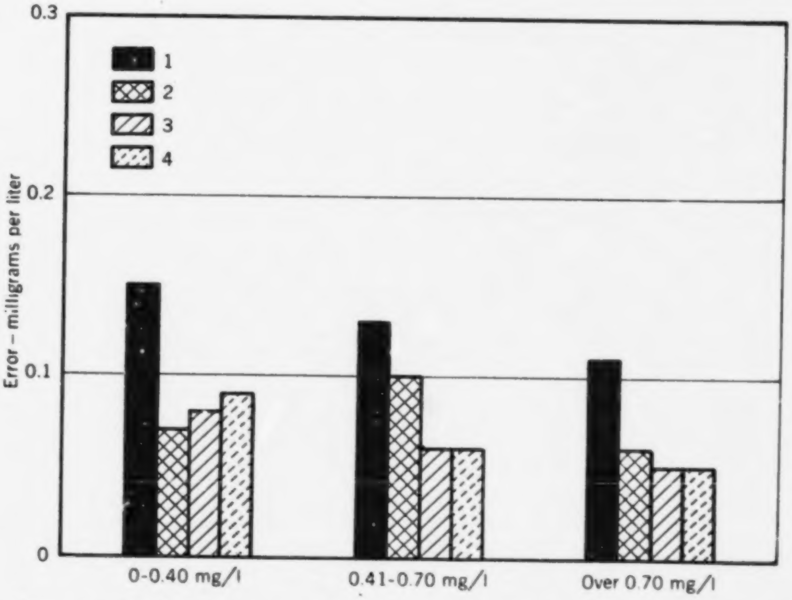

Figure 5.—*Relation of error and fluoride range to condition of reagent for Kit A. Numbers 1–4 refer to correspondingly numbered conditions of preparation and storage of reagent in text.*

The following recommendations and conclusions for Kit A are based on the results obtained in this study.

1. The specifications for the use of the reagent might be changed to permit the reaction to continue 60–90 minutes, if desired, at a temperature between 15° C and 25° C. The best combination should be determined by the manufacturer.
2. If these specifications are followed, the average error (reading higher than the actual fluoride content) should be less than 0.1 mg/1 floride.
3. Greater accuracy is obtained when the reagents are mixed by the user.
4. No differences are demonstrated with reagents stored in the dark, in light, in a refrigerator, or at room temperatures.
5. The reagent can be used for at least 6 months.

RESULTS—Kit B

Results with Kit B were opposite those obtained with Kit A—that is, in almost every reading at any time or temperature, the error was negative. When the water sample actually contained 1.0 mg/1 fluoride, the reading was less than 1.0 mg/1. Figure 6 indicates the average error under all storage conditions and light sources. There does not appear to be a significant difference in the error caused by these factors. It addition, the age of the reagent has no significant effect on the error, which is fairly constant at an average of 0.25 mg/1.

The error in the reading increases as the duration of the reaction increases, as illustrated by the average values of Figure 7. The least error occurs between the reaction times of 30 to 45 minutes, but even then it is more than 0.20 mg/1. There appears to be a slight improvement in accuracy as the age of the reagent increases.

Figure 8 indicates that this reagent should be used at higher reaction temperatures because the least error occurs between 30° C and 35° C. Here, too, the error appears to decrease slightly as the age of the reagent increases.

In figure 9 the relation of error to fluoride range is shown, as well as the effects of the various storage conditions within each range. The error apparently increases with higher fluoride values, but this may be misleading because many low samples which read less than zero, were read as zero for calculation purposes.

Conclusions on Kit B, based on data from the study, are as follows:

1. When the manufacturer's specifications are followed, the error is found to exceed the *Standard Methods* limit

of 0.1 mg/l fluoride.

2. The least error occurs when the reaction time is between 30 and 45 minutes, and at a temperature between 30° C and 35° C.

3. It is doubtful if greater accuracy is obtained with the illuminator. The difference in errors obtained when using light from the illuminator and when using natural light is not significant.

4. No differences were observed using reagents stored under various conditions, whether stored in light or dark, refrigerated, or kept at room temperature.

5. In all the participating laboratories there was considerable difficulty in matching colors. Sometimes the color match was so poor that a value could not be obtained. In addition, many of the low-fluoride samples (less than 0.4 mg/l fluoride) read less than zero so that the actual numerical error at these levels could not be obtained.

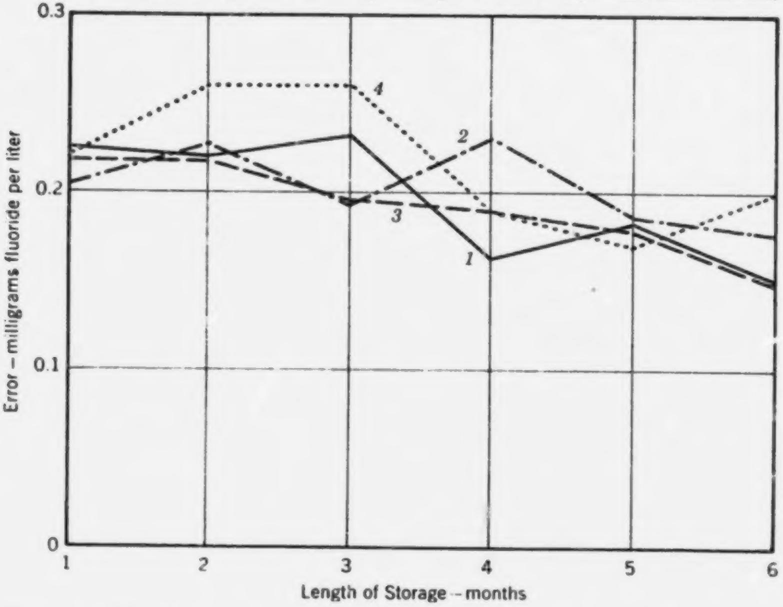

Figure 6.—*Effect of reagent storage on accuracy of Kit B. Numbers 1–4 represent the correspondingly numbered conditions of preparation and storage of the reagent referred to in the text.*

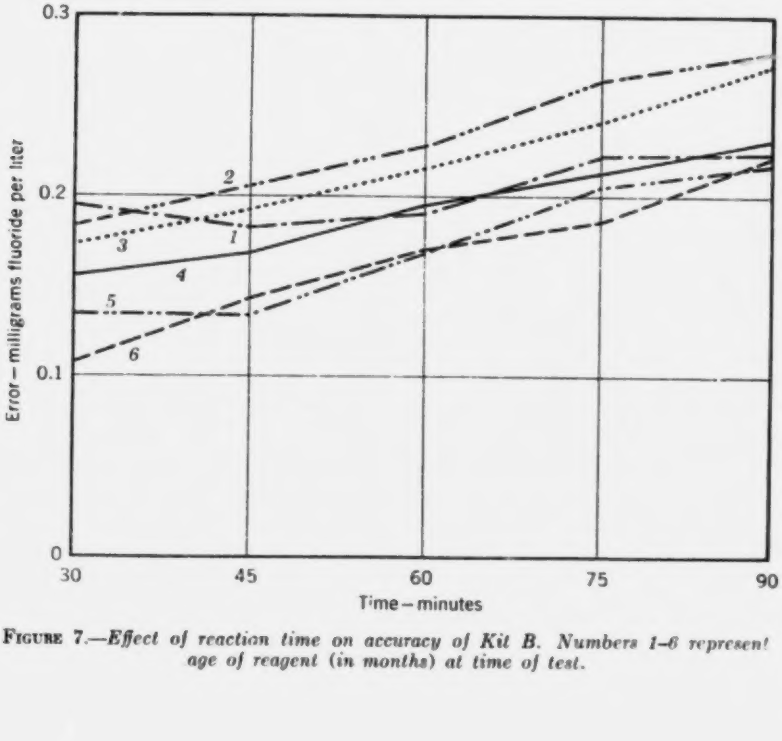

Figure 7.—*Effect of reaction time on accuracy of Kit B. Numbers 1–6 represent age of reagent (in months) at time of test.*

GENERAL CONCLUSIONS

On the basis of approximately 4,000 tests on each type of kit, it was found that the accuracy of Kit A is generally satisfactory but could be enhanced by defining more precisely the conditions of operation. Further work might also be done to improve the results obtained when the premixed reagent is used.

Kit B, although satisfactory for detecting gross fluoride feeding errors, does not produce results within the standard of accuracy defined by *Standard Methods*. Further work by the manufacturer would probably lessen this error.

ACKNOWLEDGMENTS

The following collaborated on this study, using the facilities of the laboratories indicated. Robert S. Phillips, Chairman of Task Group 2620 P, at the laboratory of the Charlotte Water Department, Charlotte, N.C., with Lee S. Dukes, sanitary engineer, and Angus Benton, chemist; Franz J. Maier, chairman of the group engaged in the study, at the USPHS Dental Public Health Laboratory, Bethesda, Md. (using Washington Suburban Sanitary Commission water) with Ervin Bellack, chemist; L. A. Smith, at the Madison Health Department laboratory, Madison, Wis., with Ervin Hindin, chemist; Ray L. Derby, at the laboratory of the Los Angeles Department of Water and Power, Los Angeles, with Clinton S. Ung, laboratory assistant, Anthony G. Macejunas, chemist, and Dorothy A. Margolin, assistant biologist; Frank S. Taylor, at the laboratory of the Oklahoma City Water Department, Oklahoma City, with John S. McReynolds, water treatment engineer.

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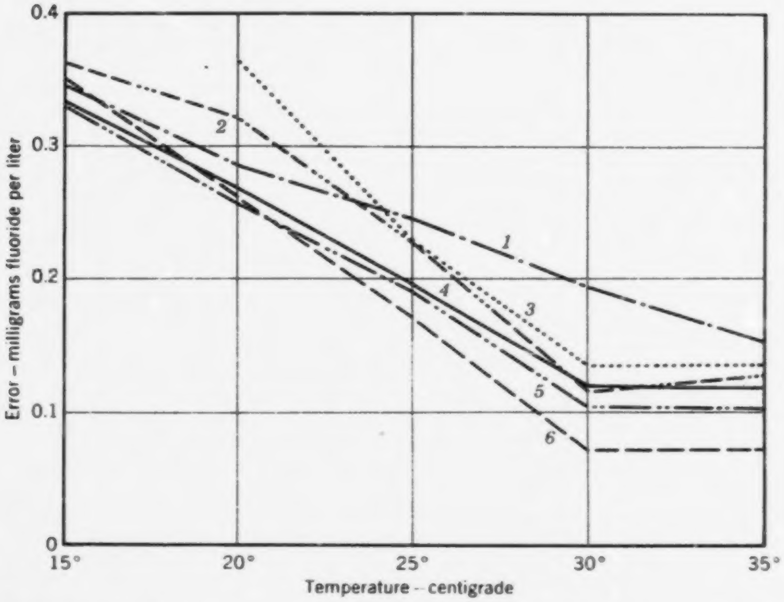

FIGURE 8.—*Effect of reaction temperature on accuracy of Kit B. Numbers 1–6 represent age of reagent (in months) at time of test.*

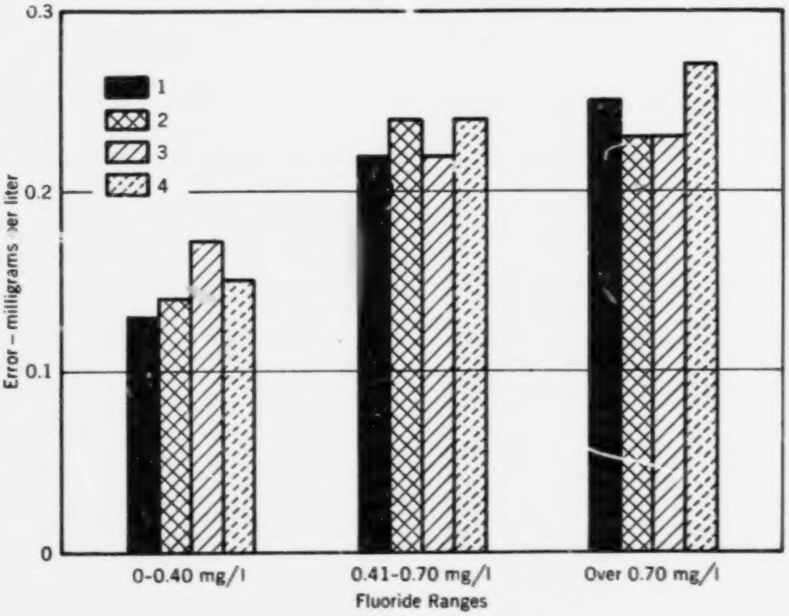

FIGURE 9.—*Relation of error and fluoride range to condition of reagent for Kit B. Numbers 1–4 refer to correspondingly numbered conditions of preparation and and storage of reagent in text.*

CONTINUOUS RECORDING OF FLUORIDE IN WATER

Franz J. Maier and Ervin Bellack

A continuous record of the fluoride concentration in water provided by an automatic analyzer would be of value to water utilities. Such a record will not only monitor the operation of the fluoride-feeding system, it would also provide a graphic history of the fluoride concentration at any point in the plant or distribution system. The recording mechanism also can be used as the primary device for actuating the fluoride feeding mechanism (*1*). In this way a constant fluoride level would be maintained automatically in the treated water regardless of variations in water consumption, fluoride level in the untreated water, and purity of the fluoride compounds.

Conductivity measurement is one of several possible principles that can be used in such a monitoring and recording system. This principle is employed now to measure the quantity of fluoride added to water (*2*). In this installation at Salem-Beverly, Mass., two conductivity cells are used; one measures the conductivity of the water before fluoridation and the other after the addition of fluorides. The difference in conductivity is then expressed in terms of the added fluoride ion by a recording apparatus. This system cannot compensate for changes in fluoride in the untreated water and consequently cannot indicate the total fluoride in the treated water. The addition or the loss of conducting ions, other than fluorides, occurring between the two conductivity measuring points would also be measured as a change in the fluoride ion concentration. Consequently, in order to utilize this principle to measure total fluorides other ions contributing to conductivity must be removed.

The development of a device for the rapid distillation of water samples to give fluoride solutions essentially free of other ions provided an approach to our problem (*3*). It was demonstrated that by regulating distillation conditions the fluoride concentration of the water sample and the distillate could be made the same. However, while the distillates from this device are free enough from interferences to allow direct chemical analysis for fluorides, they are far from satisfactory for determinations based on conductivity. Traces of sulfate ion (when sulfuric acid is used as the distillation acid) and ions resulting from volatile materials in the sample change the conductivity of the distillate to such a degree that the system is useless for fluoride measurements.

To adapt the device as a basis for continuous distillation it was necessary to determine how long the sulfuric acid could be used before its replacement was required. It was found that the only limiting factor was the total accumulation of solids in the acid bath after prolonged use. In time the bath became an almost solid mass.

The first step toward the development of a method for determining flouride continuously by conductivity measurement evolved when a quantity of distillate from the device described was redistilled without the addition of acid. Portions of this second distillate were analyzed chemically for fluoride using a Beckman Model B spectrophotometer and the ECR colorimetric reagent (*4*). Parallel conductivity measurements were made with a dip-type cell and conductivity bridge. A plot of these values revealed a direct relationship between conductivity values and fluoride concentrations.

It then became necessary to devise an apparatus which would provide such a second distillate continuously. This apparatus consisted of a Claisen flask equipped with a heater controlled by a thermoregulator connected through a mercury-plunger-type relay. The distillate from this flask was redistilled in a second flask of similar construction but without the thermoregulator. The entire double-distillation assembly, with the exception of the rubber tubing water inlet, was constructed of Pyrex glass with standard-taper, ground-glass joints. After the fluorides in the second flask had accumulated until the concentration had reached 25–30 mg/l, quantitative recovery of fluorides occurred, i.e., the fluoride concentration of the second distillate equalled that of the inlet water.

It was later observed that the fluorides in the second distillate fluctuated according to the volume of liquid in the second flask. The addition of a nonvolatile substance (glycerin) to raise the boiling point provided a means for level control by thermoregulation. However, the presence of any such substance in this flask prevented the quantitative distillation of fluorides. A sensitive level control was eventually attained by regulating the heater for this flask. Regulation was accomplished by connecting the heater through a system of relays to electrodes suspended in the flask.

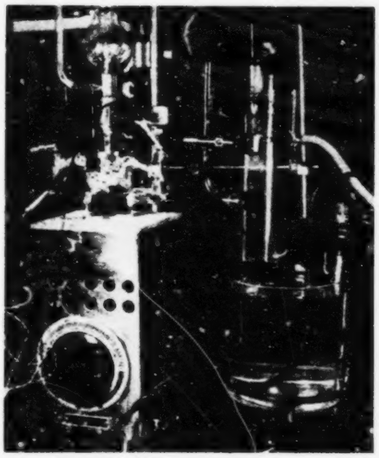

Figure 1.—*Second still and conductivity cell.*

The flask, developed for this purpose, shown in Figure 1, is of round-bottom design, 2 inches in diameter and 6 inches high. The horizontal side tubes are set as low as possible while permitting the flask to be set into the heater opening. These side tubes are of capillary size in order to restrict the passage of bubbles into the leveling chambers and are long enough to extend beyond the edges of the heater. The vertical parts of the side tubes are about one-half inch in diameter, this being the minimum size to prevent premature contact between electrodes because of condensate flow. In one side arm the platinum wire electrodes are about one-fourth inch apart, the gap being placed as low in the flask as possible to restrict the quantity of liquid in the flask. (The smaller the volume, the faster the liquid will reach equilibrium after a change in fluoride level.) In the other side arm a platinum wire extends down to a point at about the same level as the end of the lower wire in the opposite side arm.

Above the vertical tubes are connecting tubes leading back to the upper part of the flask. The size, location, and slant of these connecting tubes are such that boiling liquid will not be carried over from above and cause the liquid level to fluctuate. The 750 watt heater under the flask furnishes enough heat to insure that the second distillation will not lag behind flask A (fig. 4) which is equipped with a 550 watt heater.

The relay system for the second flask consists of two components as shown on a wiring diagram, figure 2. A cold cathode-type relay, which operates on a

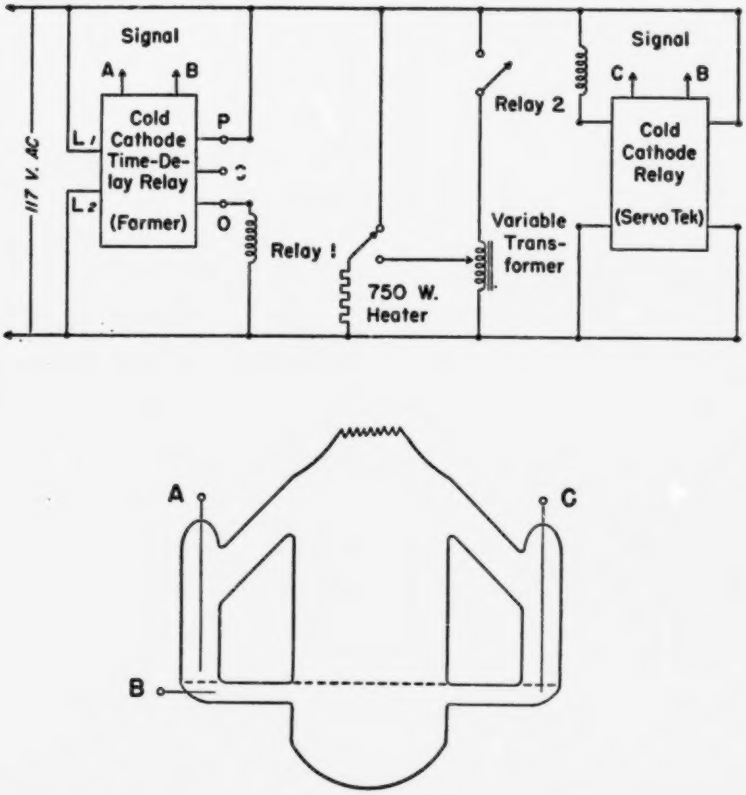

FIGURE 2.—*Relay system for second heater and position of electrodes in Second Flask.*

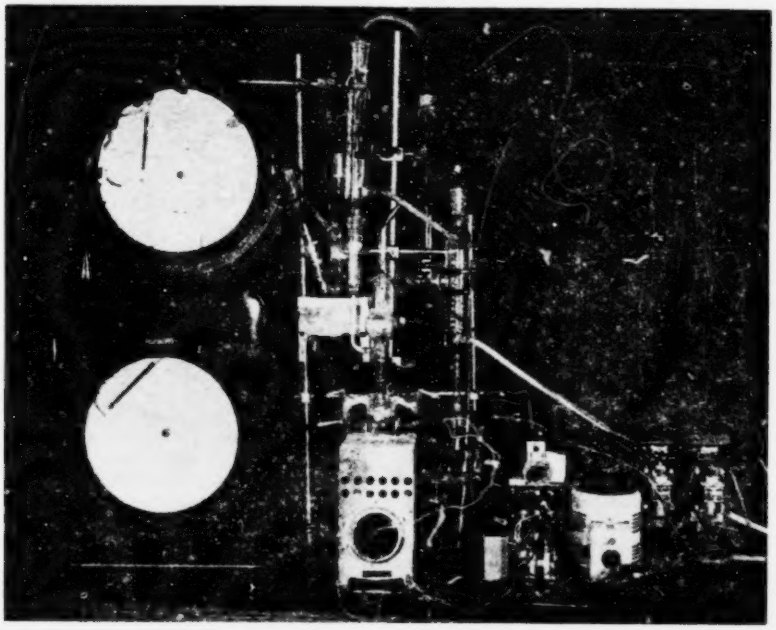

FIGURE 3.—*Fluoride conductivity assembly.*

current of 2 microamperes, triggers a relay which controls the heater. When a liquid contact exists across the gap formed by the two electrodes (A and B) in one of the side tubes, full voltage is transmitted to the heater and the distillation rate is increased. When this liquid contact is broken, the relay operates to send power to the heater through an adjustable voltage regulator. This has the effect of keeping the liquid level constant without undue cooling while the maximum heat is off. An adjustable time lag is incorporated in the relay to prevent hunting due to momentary make and break of the circuit through the liquid in the flask.

An additional circuit is utilized for a low-water cutoff and consists of the electrode in the other side tube (C), the lower electrode in the first side tube (B), and another relay. When the liquid level falls below these contacts the relay opens to cut off the heater entirely. As the liquid level rises, the heater is turned on through the above steps.

When the unit is in operation condensate from the second still flows through a modified conductivity cell (fig. 1). This cell was adapted from a dip-type cell by removing the glass shield and enclosing the plates in a U-tube equipped with the necessary vents and outlet. The elongated design permits the cell to be immersed in a constant temperature bath. The longitudinal space adjacent to the electrodes permits the insertion of a sensing element for recording temperature or for operating a temperature compensator in the conductivity recorder.

The leads from the electrodes are connected to a conductivity recorder of the resistance type (fig. 3). In this device, manufactured by the Foxboro Company, the measuring circuit is a modified Wheatstone Bridge comprising two similar air capacitors and two resistors. The sensing element is one of the resistors, while the balancing element in the recorder is one of the capacitors. As the sensing element detects a change in conductivity, the measuring circuit becomes unbalanced and sets up a voltage which after amplification causes the pen drive to operate until the measuring circuit is rebalanced.

Of all the ions present in the usual water supplies, only those which distill over from a sulfuric acid bath are likely to contribute errors to the conductivity measurements. Of these, chloride and nitrate are the most prevalent. A small amount of chloride distills over into the second still even when solid silver sulfate is added to the first still. However, as much as 400 mg in 100 ml. of solution may accumulate in the second still before any appears in the final distillate. The quantity of silver sulfate to be

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added to the first still depends on the amount of chlorides in the water, the amount of other dissolved solids (affecting the frequency of acid renewal), and the length of time the still is expected to run before the apparatus is shut down for cleaning and replacing the acid. No satisfactory method for removing nitrates has as yet been found, but nitrate also accumulates in the second still for some time before any appears in the distillate. The nitrate content of most waters is very low. Chlorine is destroyed in the first still; none has ever been found in the second. Quantitative recovery of fluoride was not prevented when water was used containing up to 2,000 mg/l sulfate, 500 mg/l alkalinity, 50 mg/l siliva, and 50 mg/l aluminum. However, when aluminum exceeded 50 mg/l the boiling temperature had to be raised above 145° C for complete fluoride recovery.

To place the unit in operation flask A in figure 4 is charged with approximately 100 ml. of 1:1 sulfuric acid, 10 gm. or more solid silver sulfate, and a few glass beads. Flask B is filled to the upper electrode with a dilute solution of fluosilicic acid in distilled water (about 20 mg/l F). Cooling water is started through the condensers and both heaters are turned on full. As soon as flask A comes up to temperature (thermoregulator is set to operate at about 140° C), evidenced by the relay operation, the water inlet is opened. When the contents of flask B begin boiling vigorously the power regulator is turned down to a point which will barely keep the solution boiling and both units are allowed to operate with their automatic controls. The recorder may then be turned on, and after sufficient water has been distilled to bring the system to equilibrium, the recorder will indicate the level of fluoride ion in the water.

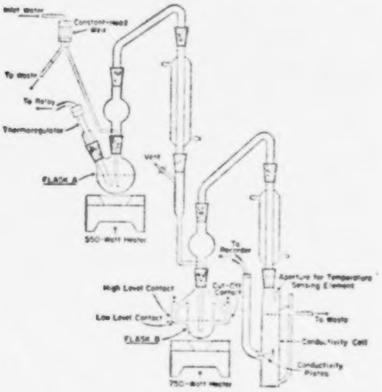

Figure 4.—*Distillation apparatus.*

Table 1.—*Conductivity*

(mmhos at 30° C)	Fluoride (mg/l)
16	0.77
17	0.83
13	0.88
19	0.93
20	0.97
21	1.00
22	1.05
23	1.08
24	1.12
25	1.15
26	1.19
27	1.22
28	1.25
29	1.28
30	1.30

Table 1 lists fluoride levels for various conductivity values.

Figure 5 shows a rather typical trace of a 24-hour run at a regulated temperature of 30° C. Figure 6 illustrates the response to changes in fluoride concentrations.

Since conductivity varies approximately 2 percent per degree C, the temperature of the condensate becomes a critical factor in determining fluoride by conductivity. If the recorder chart is to be interpreted properly, one of the following temperature compensations must be made:

1. Maintain temperature of water in the conductivity cell at a constant value and calibrate the chart accordingly.
2. Temperatures of the liquid in the cell can be read at intervals or recorded automatically in conjunction with the conductivity record. Fluoride can then be determined at any given point by use of curves applicable for various temperatures.
3. Equip recorder with automatic temperature compensation.

The latter is most satisfactory and, in this case, the recorder charts may be printed so that fluoride values may be substituted for conductivity units.

The apparatus may be allowed to operate continuously without attention, except for ink replenishment and daily chart changing, for as long as the accumulation of ions in the first still will permit. Failure to shut the still off in time will be apparent when readings begin drifting continually higher without evident cause. It would be well to limit the length of run to a definite period within a margin of safety. For water containing relatively small amounts of dissolved solids this may be

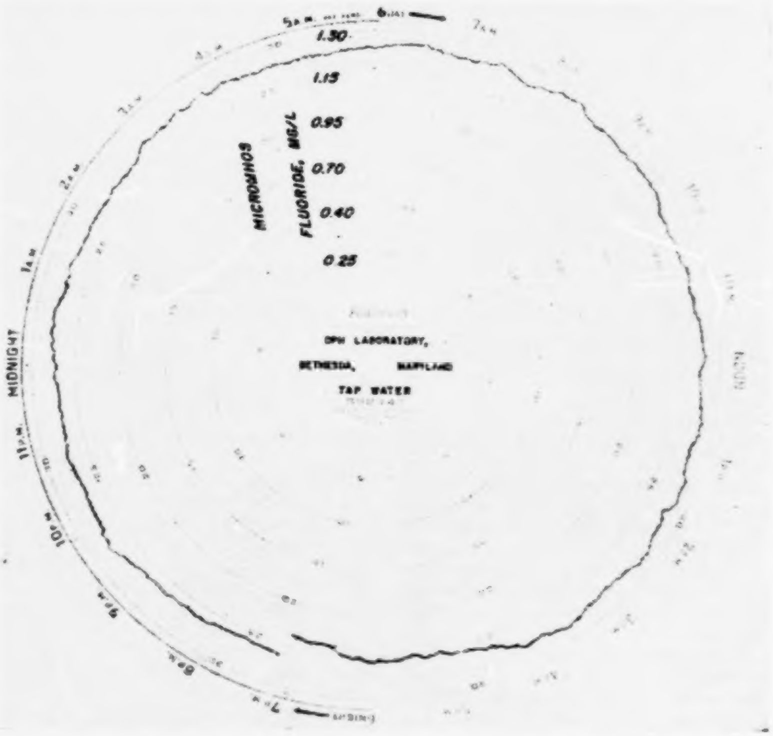

Figure 5.—*Recording chart including typical trace.*

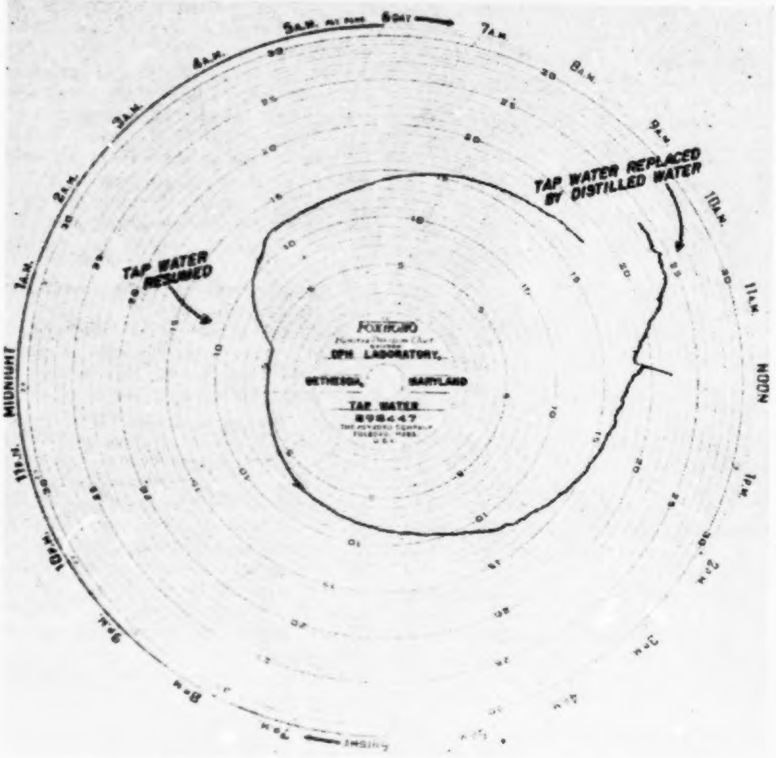

Figure 6.—*Recording chart showing response to fluoride variations.*

as long as a month, with correspondingly shorter periods for other waters. At the end of the run both heaters are turned off; the inlet water is shut off; and both flasks replaced or cleaned and recharged. The unit is then started up as before.

Among the precautions to observe during the operation of the unit are the following:

1. All joints must be tight to prevent the loss of vapor containing fluoride. A light coating of silicone lubricant on the ground-glass joints prevents freezing and permits a tight seal.

2. Electric current must be on at all times. When the current is shut off flask A will continue to fill up without distilling and flask B will be emptied by the vacuum formed by cooling of the system. In the event of power failure the inlet water should be shut off immediately and the vacuum released by opening the stopcock below the first condenser. This can be done automatically with a solenoid actuated valve.

3. A continuous flow of water must be maintained. In the event of water failure power should be turned off immediately. This can be done automatically with a pressure switch on the water line.

4. The size and frequency of swing of the recorder pen (noise) is influenced primarily by the variation in liquid level in the second still. It may be reduced by judiciously increasing the power setting until the level remains close to the upper contact at all times. However, this setting should not be so high that the full power (power regulator bypassed) does not come on occasionally.

ABSORBENCIES

The measurement of absorbencies is another principle utilized for the continuous recording of fluorides in water. With the development of an almost instantaneous colorimetric method for determining fluorides in water, the use of a color reagent and flow colorimeter became a possibility. Heretofore, all colorimetric methods of analysis required from five minutes to one hour, or more, for color development. This time lag made any truly continuous analysis practically impossible.

The ECR fluoride method produces a color which may be interpreted in terms of fluoride ion concentrations within seconds after the addition of reagents (*4*). Its use permits the employment of a flow colorimeter to measure fluoride in a virtually constantly flowing stream of water. The design of the appropriate apparatus for achieving this end is based on continuously metering the water sample and two reagents, mixing the three ingredients, and passing the mixture through an absorption cell in a photometric device. The relative absorbence or transmission of light through the cell can then be relayed to a recorder which plots these figures in terms of fluoride concentrations.

The ECR method is extremely sensitive to fluoride increments and to utilize its accuracy and precision requires precise proportioning of the sample and both reagents. First attempts with orifices calibrated to deliver the reagents in constant proportions and the water sample at a 10:1 ratio to each of them indicated that an orifice is at best a poor metering device for very low flow rates.

A truly continuous system had to be modified to that of an intermittent delivery of sample and reagents (fig. 7).

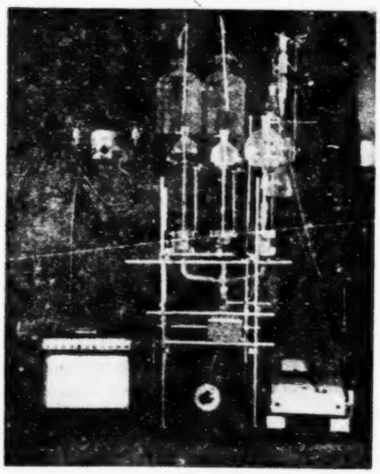

Figure 7.—*Absorption assembly including single distillation (continuous) of sample.*

Three automatic pipettes were set up so that at chosen periodic intervals 2.5 ml. of each of the reagents and 25 ml. of sample were discharged into a mixing chamber, the solution mixed for a predetermined time, and then released to flow through a colorimetric device. To make the device automatic the stopcocks on the automatic pipettes were driven by a timer-controlled electric motor and gear train so that all three pipettes would empty simultaneously. The solutions were held in the mixing chamber by a solenoid-operated pinch clamp. The solution then flowed through the cuvette of a photometer and thence to waste.

The photometers used for this purpose should have a stable circuitry and a relative narrow band pass (maximum of about 20 mμ). We have not as yet found an instrument that is ideal in both these respects. A photometer could, however, be readily designed for this specific purpose and incorporated with the rest of the apparatus into a single instrument.

The recording of fluoride level derived from any colorimeter can be made with any potentiometric recorder. We used a Brown strip-chart recorder connected through a helipot (helical potentiometer) so that the output of any colorimeter can be correlated with the full-scale deflection of the recorder pen. The recorder chart was then calibrated in terms of fluoride concentrations and the continuous recording of fluoride ion was realized.

When using this reagent on waters containing ions which interfere with the fluoride measurement, the incoming water can be distilled automatically and continuously by a device described previously for conductivity measurements. Only one distillation is necessary, however, because of the tolerance of the ECR reagents to most interferences (figure 7).

The principles utilized have been shown to be practicable with the apparatus described. Many additional methods are available for producing distillates, for proportioning reagents and for measuring absorbencies. Our apparatus can probably become simplified, more sensitive, attractively housed, and designed, and constructed to operate with less attention. We hope that some of these objectives will be accomplished at least in part in the near future.

The instrument section of the National Institutes of Health, Bethesda, Md., assisted us considerably in the development of the second distillation flask and of the circuitry required for controlling automatically the heater for this flask.

ACKNOWLEDGMENTS

Grateful appreciation is expressed to Russell H. Babcock for the use of the Foxboro conductivity recorder and to John M. Jester of the Washington Suburban Sanitary Commission for permission to test our instruments at the Patuxent filtration plant.

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SIMPLIFIED FLUORIDE DISTILLATION METHOD

Ervin Bellack

The basic technology of fluoride distillation has been little changed since Willard and Winter (*1*) reported it in 1933. Many of the conditions of distillation, including both mechanical and chemical considerations, have been thoroughly studied by a number of investigators (*2, 3*). Several modifications have been suggested with the aim of providing greater ease or less time consumption in the distillation process; among these is the use of temperature-controlling devices and compressed air to replace the steam or distilled water normally used to drive over the fluoride (*4–6*).

In spite of the improvements, the method remains somewhat cumbersome, requiring elaborate apparatus and considerable attention and skill on the part of the user. Among the drawbacks most commonly encountered are dilution of the sample and the need for careful temperature regulation during the period when steam is being introduced into the distilling flask. Some modifications (*4–6*) purport to eliminate these difficulties, but actually introduce further complications in the form of still more involved apparatus. The control of air flow rate, for example, with the pronounced effect on acid carryover and fluoride recovery, is extremely critical.

IDEAL METHOD

The ideal distillation method would be one in which there were neither steam nor compressed air used, no dilution of the water sample encountered, and no regulation of temperature required. In addition, the acid used would be one which introduced no hazard and produced no volatile ions which would interfere with the subsequent fluoride analysis. With this ideal in mind, studies were begun on an improved method which would meet some—if not all—of these requirements.

Among the contemplated improvements were the substitution of another high boiling-point acid for sulfuric or perchloric acids and the use of vacuum instead of steam or air to drive over the fluoride. Of the various materials tried as acid substitutes, only ammonium bisulfate gave any measure of promise. The temperature required to give complete recovery of fluoride was so high, however, that sulfate carryover was beyond the tolerable range and the possibility of volatilizing interfering ions was enhanced. Using an aspirator-induced air stream through the distillation flask introduced another problem—the loss of fluoride through the vacuum system. Because the possibility of finding a distillation method which would be suitable for use with such sulfate-sensitive analytical procedures as that of Megregian (*7*) seemed very unlikely, attention was diverted to a procedure which would be compatible with the *Standard Methods* analyses (*8*). Although the restrictions regarding the acid could then be relaxed somewhat, the remaining points of the ideal distillation method could conceivably be met.

The method was to be based on the principle of distilling a fixed volume from a particular sulfuric acid-water mixture so that a fixed volume of distillate would be recovered. Thus, the only control to be required of the operator was the shutting off of the still when the volume of distillate equaled the volume of sample placed in the still originally.

The remaining problem, then, was determination of the volume of acid to be employed in order to attain complete recovery of fluoride in the distillate without contamination from volatile anions.

INVESTIGATION

The study of these unknown factors was begun by investigating the disclosure by Megregian and Solet (*2*) that at least 100 ml. of distillate must be recovered at the optimum temperature range of 130°–150° C. The apparatus used consisted simply of a distilling flask, a connecting tube, and a condenser. A thermometer and the necessary adapter were included merely in order to check the progress of the distillation (fig. 1). A sulfuric acid-water mixture was prepared so that, when a 150-ml. sample was distilled, the temperature conditions would be met. Unfortunately, under these conditions, recovery of fluoride in the distillate was found to be only approximately 60 percent. Increasing the sample volume produced an increase in the recovery, but, as can be seen from the figures below, the law of diminishing returns seems to be in effect and, although an infinitely large sample would guarantee virtually complete fluoride recovery, the procedure will then have gone beyond the bound of practicality.

Boiling Point of Mixture °C	Fluoride Recovered in 200 ml of Distillate *Per cent*
155	80
160	85
165	88
170	91
175	93
180	94

Obviously, then, the conditions of temperature range which apply to conventional steam distillation do not apply here. In order to modify the temperature range, the ratio of acid to water was gradually increased, with a corresponding increase in boiling point, until further increases failed to improve the fluoride recovery. The boiling point at this stage of the experiment was 180° C and the ratio of acid to water was 2:1.

Sample Volume *ml*	Fluoride Recovered *per cent*
200	68
250	77
300	84
350	90
400	94

Increasing the volume of the acid-water mixture so that 150 ml. of distillate was recovered above 130° C resulted in a rise in the recovery to 96 percent, but further increases in the volume of the acid failed to improve recovery and, in some instances, actually lowered it. It was next demonstrated, by means of gradual increases, that 300 ml. was the minimum volume of sample with which 100 percent recovery was attainable.

Up to this point, all samples distilled were standard solutions of 1 mg. fluoride per liter of distilled water. Before the method could be seriously considered, the amount of sulfate carryover during the distillation and the amount of fluoride capable of being recovered from waters of varying fluoride levels had to be determined. The problem of contamination of successive samples when the acid mixture is reused had to be

TABLE 1.—*Results of distillation of water samples containing fluoride or fluoride and interfering ions*

Number	Composition of Sample			Fluoride Recovered *mg/l*	Mean Fluoride Recovery and Deviation *mg/l*
	Fluoride *mg/l*	Other (Interfering Ions)			
		Substance	Amount—*mg/l*		
1	1.25	Aluminum	2.5	1.27 1.22 1.23	1.24 ± 0.02
2	0.75	Hexametaphosphate	2.5	0.79 0.73 0.77	0.76 ± 0.03
3	2.00	Hexametaphosphate	10.0	2.07 2.02 2.04	2.04 ± 0.05
4	1.00	Aluminum	10.0	1.02 1.00 1.00	1.01 ± 0.01
5	1.20	Aluminum	2.5	1.31 1.27 1.24	1.27 ± 0.03
6	0.75	Hexametaphosphate	7.5	0.80 0.71 0.76	0.76 ± 0.04
7	0.50	—	—	0.51 0.49 0.49	0.50 ± 0.01
8	3.00	—	—	2.88 2.94 2.92	2.91 ± 0.09
9	0.09	Hexametaphosphate Sulfate	5.0 500.0	0.10 0.08 0.10	0.09 ± 0.01
10	10.0	—	—	9.80 9.60 9.70	9.70 ± 0.30
11	0.20	—	—	0.23 0.21 0.21	0.22 ± 0.02
12	0.95	Aluminum	20.0	0.97 0.95 0.92	0.95 ± 0.02

solved and the effect of volatile anions, the effect of ions which might retard recovery, and the limits of the distillation rate had to be determined.

To ascertain the amount of sulfate carryover, a series of distillates were checked and found to contain less than 40 mg./l in any sample—with about 30 mg./l being most typical. This would then permit the use of any of the standard fluoride methods (8) with practically no sulfate interference. For recovery information, a number of water samples—some containing interfering ions—were distilled in triplicate. Results indicated that fluoride recovery was virtually complete on all samples, regardless of fluoride level (table 1). This series of samples, although intended to demonstrate fluoride recovery at different levels, also serves to indicate the lack of interference from ions which normally would affect the fluoride determination. Thus, aluminum in as high a concentration as 50 mg./l, hexametaphosphate to 25 mg./l, and phosphate to 50 mg./l, do not interfere with fluoride determination in the distillate. The lack of contamination from successive samples of different fluoride levels can also be shown here because the samples were run in the order indicated without changing acid or flushing the still. The only exception to this occurred following sample 10, which contained 10 mg./l fluoride. It was found necessary to flush out the still with distilled water before the next sample when a trial low-fluoride sample, distilled without prior flushing, produced results approximately 0.1 mg./l too high. It can be seen, however, that samples with as high as 3.0 mg./l fluoride can be distilled without fear of contaminating the following sample.

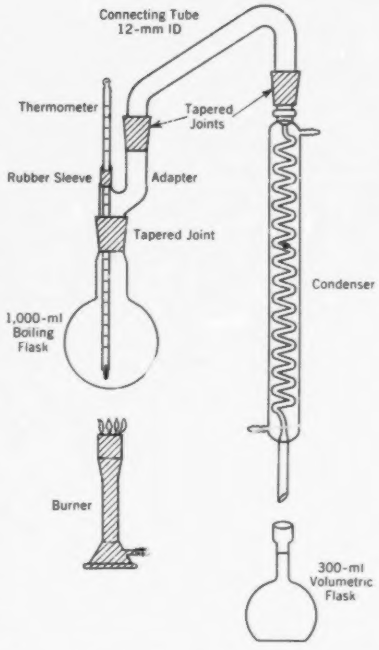

Figure 1.—*Distillation apparatus. Thermometer and adapter may be omitted in routine procedure.*

Further tests indicated that up to 300 mg./l aluminum (90 mg. in the sample), 400 mg./l silicon dioxide as colloidal silica (120 mg. in the sample), and 300 mg./l silicon dioxide as silicate (90 mg. in the sample) did not retard the recovery of fluoride. As could be expected, the effect of large quantities of chloride caused interference in the analysis, but the use of silver sulfate in the distilling flask at a proportion of 5 mg./mg. of chloride effectively reduced to a minimum the hydrochloric acid carryover into the distillate.

Table 1.—*Results of distillation of water samples containing fluoride or fluoride and interfering ions*—Continued

Number	Composition of Sample			Fluoride Recovered *mg/l*	Mean Fluoride Recovery and Deviation *mg/l*
	Fluoride *mg/l*	Other (Interfering Ions)			
		Substance	Amount—*mg/l*		
13	1.10	Aluminum	50.0	1.08 1.10 1.10	1.09 ± 0.01
14	0.85	Hexametaphosphate	25.0	0.88 0.85 0.87	0.87 ± 0.02
15	0.75	Phosphate	37.5	0.81 0.78 0.79	0.79 ± 0.04
16	0.22	Phosphate	50.0	0.24 0.23 0.23	0.23 ± 0.01

The time required for distillation proved to be simply a matter of the efficiency of the condenser and the capacity of the burner. The usual amount of time consumed in distilling a 300-ml. sample was 15-20 minutes. By using two burners, the distillation time could be reduced to as little as 10 minutes, although by doing so the sulfate carryover was increased somewhat.

APPARATUS

The original apparatus design proved to be quite satisfactory and any modification would have to be carefully considered. The apparatus used was the following: one 1-liter, round-bottom, long-neck, pyrex boiling flask (standard taper joint, 24-40 mm.); connecting tube, (joints as in the preceding item); one long, efficient Graham condenser; one receiver calibrated at 300 ml.; one thermometer with scale to 200° C and adapter, both of which may be omitted for routine use; and one burner. All apparatus was manufactured by Corning Glass Works, Corning, N.Y., except the receiver which is a product of the Arthur H. Thomas Co., Philadelphia, and the thermometer and burner, both products of the Fisher Scientific Co., Pittsburgh, Pa.

The primary considerations are based on conditions which could affect the complete recovery of fluoride—such as obstruction in the vapor path, trapping of liquid in the adapters and condenser, and conditions which might enhance sulfate carryover.

PROCEDURE

The procedure for the distillation is as follows: Place 400 ml. distilled water, 200 ml. concentrated sulfuric acid, and 2-3 dozen glass beads in the distilling flask and swirl to mix. Connect the apparatus as shown in figure 1, making sure all joints are tight. Begin heating slowly at first, then as rapidly as the efficiency of the condenser will permit (distillate must be cool). Heat until the temperature of the flask contents reaches 180° C. At this point, the apparatus has been flushed out and the acid-water ratio has been adjusted. As soon as the flask contents cool to 120° C—in about 20 minutes—the apparatus is ready for the sample. Exactly 300 ml. of sample is added to the flask, mixed, and the distillation carried out as before until exactly 300 ml. of distillate is recovered.

Occasional checking of the temperature at the end of the distillation will serve to verify the accuracy of measurement of the sample and distillate. The thermometer need not be used routinely once the operator is sure that the distillation ends at 180° C each time. The still need not be flushed out except when samples containing more than 3.0 p.p.m. of fluoride have been distilled. At those times, a 300-ml portion of distilled water should be distilled through the apparatus to prevent contamination of successive low-fluoride samples.

The acid need not be replaced until the accumulation of ions causes carry-over of interferences or retards fluoride recovery. An accumulation of 300 mg. of aluminum or 300 mg. of silicon dioxide as silicate will affect subsequent fluoride recovery. An occasional recovery check with standard fluoride samples will indicate when the acid is to be replaced. When fresh acid is used, the procedure outlined at the beginning of this section should be followed. When water samples with high chloride content are to be distilled, silver sulfate, in the proportion 5 mg./mg. of chloride, should be added to the distilling flask.

CONCLUSION

A distillation method for fluoride has been found which requires neither steam nor compressed air and in which no dilution of the water sample is encountered. The principle involved is the distillation of a volume of water sample from a particular sulfuric acid mixture so that the same volume of distillate—with full fluoride recovery—is obtained. The method is simple, requires only standard items of apparatus, and consumes very little time.

ACKNOWLEDGMENT

The assistance provided by Paul J. Schouboe in the distillation and analysis of water samples is gratefully acknowledged.

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RAPID PHOTOMETRIC DETERMINATION OF FLUORIDE IN WATER. Use of Sodium 2-(*p*-Sulfophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonate-Zirconium Lake

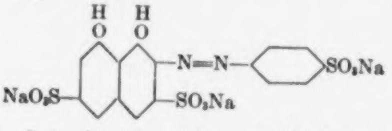
ERVIN BELLACK and P. J. SCHOUBOE

A simple and rapid technique for determining fluoride in water samples gives an accuracy within 0.02 mg. per liter in the fluoride concentration range of 0.00 to 1.40 mg. per liter. Its speed and relative tolerance to interfering ions make it a potential substitute for present alizarin-zirconium photometric methods, as well as for spectrophotometric methods requiring special devices for handling interfering ions.

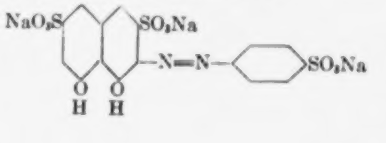
Water samples are usually analyzed for fluoride by one of the several alizarin-zirconium methods, either visually or by using a photoelectric colorimeter (*1*). These methods have one serious drawback in that color development is progressive, requiring accurate timing to achieve consistent results. The waiting-period is also a source of constant annoyance. Recently, more rapid methods have been developed, but these usually require the use of a spectrophotometer and are particularly sensitive to certain interfering ions (*7, 9*). This sensitivity necessitates distillation for most determinations, or, as an alternative, requires an extra step for precipitation of, or compensation for, the interference.

The method proposed can be applied directly to most water samples without prior distillation or other pretreatment, giving fluoride values with precision and accuracy with no waiting period. Because the reagent has high sensitivity to fluoride ions over a comparatively broad range of wave lengths, either a spectrophotometer or a filter photometer can be used for the determination.

Sodium 2-(*p*-sulfophenylazo)-1,8-dihydroxynaphthalene - 3,6 - disulfonate (SPADNS) has appeared in the literature as a zirconium and thorium reagent, and also as an indicator in the thorium titration of fluorides (*2–4,6*). A search of the literature revealed no previous record of the use of the SPADNS-zirconium lake in the direct photometric determination of fluoride in water samples. The structural formula of SPADNS is given as:



It is also referred to as 4,5-dihydroxy-3 - (*p*-sulfophenylazo) - 2.7 - napthalenedisulfonic acid trisodium salt (Eastman Organic Chemicals, No. 7309):



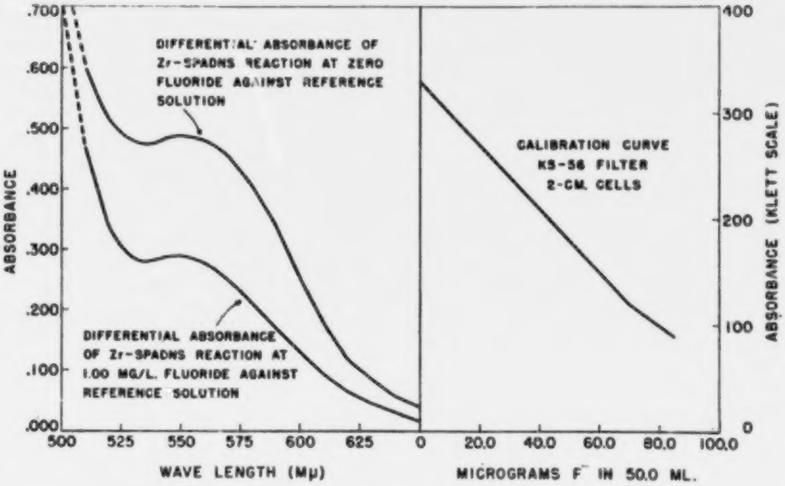


FIGURE 1.—*Absorption spectra and standard curve for zirconium-SPADNS reaction.*

PRELIMINARY STUDIES

The objectives for the proposed reagent were the following:

Rapid reaction with fluoride ions plus stability at equilibrium.

Maximum sensitivity to fluoride ions within a broad range of fluoride concentration.

Minimum sensitivity to other ions normally found in potable water, so that most water samples can be analyzed without pretreatment.

A color range which can be accurately measured with a filter photometer, as well as with a spectrophotometer—i.e., sensitivity to fluoride extending over a comparatively broad range of wave lengths.

A simple procedure.

Some compromise regarding the range of fluoride sensitivity had to be made in order to confine the range to that portion obeying Beer's law, but otherwise the proposed method satisfactorily conformed to the above objectives.

Previous studies revealed that reaction rate between fluoride ion and zirconium-dye complexes is influenced greatly by acid concentration, the reaction being almost instantaneous in the region of 0.7 *N* hydrochloric acid. Also, at this acidity the interferences of alkalinity, chloride, and ferric iron are largely eliminated and the sensitivity to fluoride ion is increased. On the basis of Megregian's experiments with Eriochrome Cyanine R, a molar ratio of 1 zirconium to 4 of dye was selected for preliminary studies (*7*).

Apparatus.—Beckman Model B spectrophotometer, or equivalent, and 10-mm. matched cuvettes, or Klett-Summerson photoelectric colorimeter, or equivalent, with rectangular cuvette having 2-cm. light path.

Preparation of Reagent.—Reagent A. SPADNS (0.958 gram) is dissolved in distilled water and diluted to 500 ml.

Reagent B. Zirconyl chloride octahydrate (0.133 gram) is dissolved in about 25 ml. of distilled water. To the zirconium solution are added 350 ml. of concentrated hydrochloric acid (reagent grade, sepecific gravity 1.19), and the mixture is diluted to 500 ml. with distilled water. Equal volumes of Reagents A and B are combined to produce a single reagent.

Reference Solution.—Ten milliliters of Reagent A are added to 100 ml. of distilled water, and to this are added 10.0 ml. of a solution containing 7.0 ml. of concentrated hydrochloric acid diluted to 10.0 ml. with distilled water. This solution is used for setting the reference point (zero) of the spectrophotometer or colorimeter; it is stable and may be reused indefinitely.

Preparation of Standard Curve.—The standard curve is prepared by subjecting standard solutions of fluoride to the following procedure and plotting the absorbance values at a standard temperature.

Standards should be selected in the range of 0.000 to 0.070 mg. of fluoride in 50.0-ml. aliquots (0.00 to 1.40 mg. per liter). As the resultant curve is a straight line of negative slope between these figures, fluoride value can be calculated on the basis of a pair of standards run concurrently with the unknown sample. Where A_0 is the absorbance of a 0.00 fluoride standard, A_1 is the absorbance of a 1.00 mg. per liter standard, and A^x is the absorbance of the unknown sample, the fluoride value in milligrams per liter of the sample is:

$$X = \frac{A_0 - A_x}{A_0 - A_1}$$

Calculating fluoride values on the basis of at least one pair of standards per day eliminates, to some degree, the necessity for adjusting the temperature of the sample to that of the standard curve, as normally both standards and sample will be at room temperature. Also, any slight aging effect of the reagent will be taken into account.

Procedure for Water Sample.—If the sample contains free chlorine, two drops of 0.1*N* arsenite solution are added for each milligram per liter of chlorine present. If other interfering ions are present in sufficient quantity to produce an error of 0.1 mg. per liter or more, or if analysis is unknown, the sample should be distilled. A 50.0-ml. water sample, or aliquot diluted to 50.0 ml., containing no more than 0.070 mg. of fluoride (1.40 mg. per liter), is adjusted to the temperature of the standard curve or standards (±2° C.). Ten milliliters of mixed reagent, or 5 ml. each of Reagent A and B, are added to the sample and mixed well.

The spectrophotometer is set at zero absorbance at 570 mμ with the reference solution, or the colorimeter is zeroed similarly using a filter having a transmittance range of 540 to 590 mμ (Klett No. KS-56 or equal). The absorbance reading of the sample is taken immediately or at any subsequent time. If the absorbance falls beyond the range of the standard curve or calculates to be higher than 1.4 mg. per liter, the fluoride concentration is too high. In this case, the procedure must be repeated using a smaller sample aliquot.

If the presence of aluminum ion is suspected, the reaction is permitted to continue for an additional 15 minutes and another reading is taken. An appreciable drop in absorbance indicates the presence of aluminum ion as an interference. At this point, holding the reacted sample for 2 hours before making the absorbance reading will eliminate the interference effect of up to 3.0 mg. per liter of aluminum; for 4 hours, up to 30.0 mg. per liter.

Interference.—Water samples subjected to distillation by either the Willard and Winter (*10*) method or Bellack (*5*) modification can be analyzed directly by this procedure, with no neutralization, concentration, or buffering required.

TABLE I.—*Effect of interfering substances on fluoride determination*

Interfering Substance	Concentration, Mg./L.	Effect on F Reading at 1.00 Mg./L.	
		Increase, mg./l.	Decrease, mg./l.
Acidity	3000	0.1	
Alkalinity (as $CaCO_3$)	5000		0.1
Al^{+++}	0.1		0.1[a]
Arsenite	1300	0.1	
Ca^{++}	800	0.1	
Cl^-	7000	0.1	
Cl_2 (should be completely eliminated)	...	If not removed[b]	
Color (should be low or compensated for)	...	May do either	
Fe^{+++}	10.0		0.1
Mg^{++}	1250	0.1	
Mn^{++}	40	0.1	
PO_4^{---}	16	0.1	
$(NaPO_3)_6$	1.0	0.1	
SO_4^{--}	200	0.1	
Turbidity (should be clear)	...		

[a] Figure for immediate reading. Allowed to stand 2 hours, tolerance is 3.0 p. p. m.; 4-hour tolerance is 30.0 p. p. m.

[b] Reduce with arsenite.

TABLE II.—*Fluoride determination in water samples*

(Values given in milligrams per liter)

Sample	Composition			SPADNS	Megregian-Maier
Synthetic					
A	F^-	1.20		1.22	
	Alkalinity (as $CaCO_3$)	150		1.20	
	SO_4^{--}	50		1.19	
	Na^+	95	Av.	1.20	
B	F^-	0.75		0.76	
	Cl^-	100		0.75	
	PO_4^{---}	2.0		0.76	
	Na^+	67	Av.	0.76	
C	F^-	0.50		0.50	
	Al^{+++}	0.05		0.49	
	Fe^{+++}	2.0		0.49	
	Cl^-	4.0	Av.	0.49	
	Na^+	0.6			
Unknown					
A	...			1.08	1.05
B	...			1.10	1.09
C	...			0.99	1.01
D	...			1.09	1.14
E	...			0.95	0.96
F	...			1.15	1.11

For water samples which are subjected to direct analysis the tolerance to various interferences is shown in table I. The error produced by a given quantity of interfering ion is not proportional to the quantity, so no mathematical compensation for interference may be made. Whenever the amount of interfering ion present is unknown, or any single ion or combination of ions is present in quantity great enough to produce an error of 0.1 mg. per liter or more, the sample must be distilled.

Temperature Effect.—The determinations may be carried out at any convenient temperature within the range of 15° to 30° C. The important consideration here is that standards and sample be at nearly identical temperature, because an error of approximately 0.01 mg. per liter of fluoride is caused by each degree difference in temperature.

ANALYTICAL DATA

Reagent Stability.—Batches of reagents including samples of A alone and B alone, as well as the two mixed, were subdivided into several lots and stored under various conditions of light and temperature. Temperature varied from 14° to 30° C. and light varied from total darkness to normal room lighting. After 3 months of storage, no perceptible changes in absorbancy or sensitivity were evident under any of these conditions.

Water Samples.—To test the applicability of the method to water samples, a number of synthetic waters, prepared from distilled water and analytical grade chemicals, were analyzed in triplicate. In addition, unknown water samples received for routine analyses were subjected to parallel testing by both the SPADNS method and that currently in use in the laboratory (Megregian-Maier method) *(8)*. Results of these tests (table II) showed excellent accuracy and precision on the synthetic waters, and good agreement between the methods on the unknown samples.

SELECTION OF REACTION CONDITIONS

The reaction conditions investigated were acidity, ratio of dye to zirconium, quantities of dye and zirconium, and the optimum wave length for use.

As mentioned previously, 0.7*N* hydrochloric acid was chosen as the reaction acidity for preliminary studies. Varying the acidity from 0.2*N* to 0.9*N* revealed that the greatest sensitivity for fluoride was attained at 0.5*N*. However, at this point the interference caused by certain ions, particularly sulfate, was enhanced. Therefore, 0.7*N* was selected as the acidity giving the best compromise between sensitivity and tolerance to interferences.

Over the considerable range of dye to zirconium ratios studied, [illegible] to 1 provided the greatest fluoride sensitivity.

Quantities of dye and zirconium, in an acid medium of 0.7*N* hydrochloric, at the ratio described above, were also varied in an effort to extend the range of measurement and to provide the maximum sensitivity to fluoride. The optimum quantities yielded a reagent which followed Beer's law from 0.00 to 1.40 mg. per liter of fluoride. When quantities were varied to extend this range, sensitivity to fluoride or tolerance to interference decreased.

In figure 1 are presented curves for the differential absorbance of the zirconium-SPADNS reaction with 0.00 and 1.00 mg. per liter of fluoride. The maximum differential occurs at 570 mμ; therefore, this wave length was chosen for the spectrophotometer setting.

As the sensitivity to fluoride extends over a comparatively broad range of wave lengths, most glass filters having peak transmittancy near the optimum wave length can also be used. One of these, the Klett KS-56, was used in these experiments.

SUMMARY

A rapid photometric determination of fluoride ion concentration uses the reactions between zirconyl ions and SPADNS for color formation, followed by decolorization of the lake by fluoride ions. The reaction is immediate, stable, and follows Beer's law in the concentration range of 0.00 to 1.40 mg. of fluoride per liter. This method, used in place of the usual alizarin-zirconium colorimetric methods, will save considerable time and increase precision. It may be applied directly to most water samples without any pretreatment.

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FURTHER STUDY OF PERMANENT COLOR COMPARATOR FOR FLUORIDES

By Franz J. Maier and Task Group 2620 P, AWWA

A study of two permanent color comparators for fluorides was published in the April 1957 issue of the Journal (*1*). Among the conclusions mentioned in the report was that the kit manufactured by W. A. Taylor Co., Baltimore, Md.:

> . . . although satisfactory for detecting gross fluoride feeding errors, does not produce results within the standard of accuracy defined by *Standard Methods*. Further work by the manufacturer would probably lessen this error.

MODIFIED TESTING KIT

Since the publication of the 1957 report by AWWA Task Group 2620 P—Fluoridation Materials and Methods, the manufacturer has made available a modified version of its fluoride testing kit. The modifications include a new slide containing permanent color standards in vials, longer analyzer tubes, and the use of the Scott-Sanchis reagent, which supplants the one developed by Lamar. The new tubes and slide fit and are intended to be used in the older "water analyzer" apparatus.

With the announcement of the availability of this modification, AWWA furnished the new parts and reagents to each member of the original task group, which was to evaluate the new kit. The purpose of this study was to determine, as before, the conditions that would produce optimum accuracy in the fluoride determination and also the factors that could produce results more inaccurate than those obtainable from the procedures found in *Standard Methods* (*2*).

LABORATORY TESTS

On the basis of information obtained from the earlier study, a reexamination of the effects of reagent storage conditions and reagent life was not deemed necessary. Similarly, the effects of artificial illumination were not reinvestigated. The only points considered in this study were the effects of reaction times and sample temperatures when they varied from those prescribed by the manufacturer.

The procedure adopted was standardized for the participating laboratories. The water samples tested were those being routinely examined. In each of three successive portions, the first was run undiluted, the second was diluted one-half, and the third diluted one-fourth. The necessary volume of water for each sample was collected in one container, mixed, dechlorinated if necessary, and divided into portions for temperature evaluation and time readings, as shown on the reporting form (Fig. 1). Those portions used for temperature evaluation were brought to, and kept at, the required temperature throughout the reaction period by means of water baths. The single portion needed for the time readings generally was the one prepared for the 25° C. temperature test.

As shown on the reporting form, the samples were examined at various times (±2 min.) and temperatures (±1° C.). The manufacturer's recommended reaction time and temperature range are also shown on the form.

Each laboratory completed only one form, generally within 1 month's time. This represents the examination of ten samples, with ten readings made for each sample. It also includes ten additional readings by means of the *Standard Methods* technique preferred by the laboratory.

In the calculation of the average error of the readings, the gross error for each analysis was computed by obtaining the difference between the *Standard Methods* results and those obtained with the kit. The data plotted in Figures 2 and 3 are arithmetical averages (obtained from Table 1) of these deviations without respect to sign.

RESULTS

In contrast to the earlier study, during which each kit tended to give either consistently high or consistently low readings, the kit tested gave readings that varied from the *Standard Methods* results in both directions. For instance, when the water samples contained 1.0 p.p.m. fluoride, readings both higher and lower than 1.0 p.p.m. were reported by the different people doing the testing.

Figure 2 (left) shows that the optimum temperature is close to 25° C. In the range 23–27° C., there is little difference in error, but at temperatures out-

TAYLOR COLOR COMPARATOR FOR FLUORIDE

Laboratory________________

Analyst________________

(Taylor Instructions: "Exactly 1 hr," 20–30°C)

Sample	Standard Methods	Time (±2 min)					Temperature (±1°C)				
	F *mg/l*	30	45	60	75	90	15	20	25	30	35
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											

FIGURE 1.—*Form Distributed by AWWA Task Group. In the column labeled "Standard Methods," the concentration of fluoride determined with the procedure from* Standard Methods *was recorded. The concentrations obtained with the Taylor kit were recorded in the other columns.*

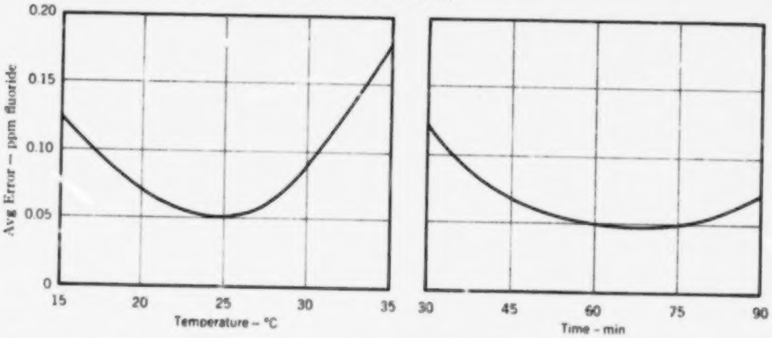

FIGURE 2.—*Effects of Temperature and Time. The curve at the left shows that the optimum temperature is about 25° C.; the curve at the right shows that the duration of the reaction could exceed 60 min.*

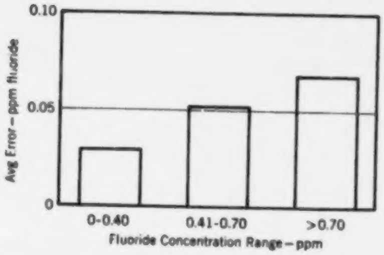

FIGURE 3.—*Relationship of Error to Fluoride Concentration Range. Greater error was found in the higher concentration range, although the percentage error over the entire range was relatively constant.*

side this range, the error increases rapidly. Figure 2 (right) shows that the duration of the reaction could exceed 60 min. without danger, although reaction times shorter than about 60 min. and longer than 75 min. introduce an excessive error. Figure 3 shows the relationship of error to fluoride concentration range. A definite trend toward greater error in the higher fluoride concentration range is evident, although the percentage error over the entire range remains relatively constant.

RECOMMENDATIONS AND CONCLUSIONS

On the basis of the results obtained in this study, the instructions for using the kit might be changed to permit the reaction to continue 60–75 min., if desired, at a temperature of 23–27° C. If these specifications are followed, the average error should be less than 0.1 p.p.m. fluoride.

This study, although limited in scope, has indicated that the modified kit is superior to the earlier model tested. In contrast to the relative inaccuracies shown by the earlier model, the modified kit should produce results approaching the standard of accuracy suggested in *Standard Methods,* when the manufacturer's recommendations are followed. Better accuracy should be attained by: (1) a more precise definition of the conditions of operation and (2) making the intervals between successive fluoride concentrations on the color standards slides closer together—namely, 0.1-p.p.m. intervals rather than 0.2-p.p.m. intervals Additional improvements that might be considered include:

1. Comparison tubes should be of better optical qualities.
2. The tubes should be provided with a means for more positive positioning within the holder.
3. The illuminator, although not absolutely necessary, could be improved by a more rigid support for the light holder.
4. A spacer could be provided for maintaining a fixed distance between the light source and the tubes.

ACKNOWLEDGMENTS

The following collaborated in this study using the facilities of the laboratories cited: Ray L. Derby, at the laboratory of the Los Angeles Department of Water and Power, Los Angeles, with Anthony G. Macejunas, chemist; Ervin Hindin, sanitary chemist at the Division of Industrial Research Laboratory, Washington State University, Pullman, Wash.; F. J. Maier, chairman of Task Group 2620 P, at the USPHS Dental Public Health Laboratory, Bethesda, Md. (using Washington Suburban Sanitary Commission water), with Ervin Bellack, chemist; Robert S. Phillips, at the lab-

oratory of the Charlotte Water Department, Charlotte, N.C., with Lee S. Dukes, sanitary engineer, and Angus Benton, chemist; Frank S. Taylor, at the laboratory of the Lake Hefner Water Treatment Plant, Oklahoma City, Okla., with Charles T. Gordon, chemist; and Harry W. Tracy, at the laboratory of the San Francisco Water Department, Milbrae, Calif., with Harold J. Lewis and Matthew J. Nunan Jr., chemists.

Table 1.—*Summary of Results From Laboratories.*

Laboratory Number	Average Error With Time—*min*					Average Error With Temperature—°C					Average Error With F Range at 25°C (60 min)* *ppm*		
	30	45	60	75	90	15	20	25	30	35	0–0.40	0.41–0.70	>0.70
	Average Weighted Error—*ppm fluoride*												
1	0.08	0.02	0.05	0.07	0.10	0.11	0.06	0.05	0.08	0.12	0.025 (8)	0.100 (4)	0.050 (8)
2	0.147	0.067	0.027	0.017	0.028	0.123	0.048	0.027	0.087	0.167	0.027 (8)	0.010 (4)	0.035 (8)
3	0.060	0.045	0.020	0.045	0.055	0.125	0.040	0.025	0.050	0.155	0.008 (6)	0.033 (6)	0.025 (8)
4	0.05	0.046	0.046	0.04	0.141	0.05	0.04	0.04	0.046	0.161	0.010 (6)	0.047 (6)	0.065 (8)
5	0.06	0.05	0.07	0.07	0.12	0.19	0.12	0.06	0.06	0.31	0.033 (6)	0 (2)	0.092 (12)
6	0.26	0.18	0.09	0.045	0.040	0.08	0.08	0.09	0.17	0.27	0.050 (6)	0.083 (6)	0.125 (8)
Average (weighted)	0.11	0.07	0.05	0.05	0.08	0.11	0.07	0.05	0.08	0.18	0.026 (40)	0.051 (28)	0.067 (52)

* The number of samples is shown in parenthesis.

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AUTOMATIC FLUORIDE DISTILLATION

By Ervin Bellack

Distillation of water samples prior to fluoride analysis, although often a necessary procedure, has remained a disagreeable laboratory operation. The time consumed, the potential hazard, and the unreliability of results have been contributing factors toward making the operation objectionable. With the recent modifications in the distillation method, some of the objections have been overcome, but the procedure still is not entirely foolproof (*1–6*).

DIRECT DISTILLATION METHOD

The direct distillation method, however, lends itself readily to automation, and thus even routine water samples can be distilled without appreciable effort on the part of the analyst (7). In this method, a sulfuric acid mixture is prepared by mixing 400 ml. of distilled water with 200 ml. of concentrated sulfuric acid and distilling until a temperature of 180° C. is reached. Sample distillation is accomplished by adding a 300-ml. sample to the mixture and again distilling to the same endpoint.

AUTOMATIC OPERATION

Basically, as the distillation is completed when the temperature of the reaction mixture reaches 180° C., conversion to automatic operation requires only that a thermoregulator replace the thermometer. The thermoregulator can then be used to activate the necessary mechanism to shut off the heat when the correct temperature is reached. The simplest arrangement would utilize an electric heater, controlled through a relay. Gas heat could also be used if the gas supply were interrupted by a solenoid valve or solenoid-operated pinch clamp. Further refinements are required, however, to eliminate the necessity for mixing the sample and acid, and also to make possible the addition of the sample without dismantling the apparatus. These refinements have been incorporated in the apparatus shown in Figure 1. It consists of a 1-liter, round-bottom, three-neck flask; Claissen adapter; funnel; inlet tube; connecting tube; and condenser. Heat is supplied by a quartz mantle, and the entire assembly rests on a magnetic stirrer. The mantle is controlled by a thermoregulator and a system of relays. When the push-button switch is depressed, initiating the distillation, heat is applied until the temperature reaches 180° C., and is then turned off automatically. The same relays also control the magnetic stirrer. The control circuit is shown in Figure 2.

PROCEDURE

Prepare the flask exactly as stated in *Standard Methods* but substitute a glass or plastic-covered stirring bar for the glass beads. The stirring bar was covered with Teflon, made by E. I. du Pont de Nemours & Co., Wilmington, Del. Adjust the thermoregulator to cut off at about 180° C. It may be necessary to set the thermoregulator several degrees lower than 180° C. because of the lag in the heating mantle. In any event, the distillation should cease when the flask

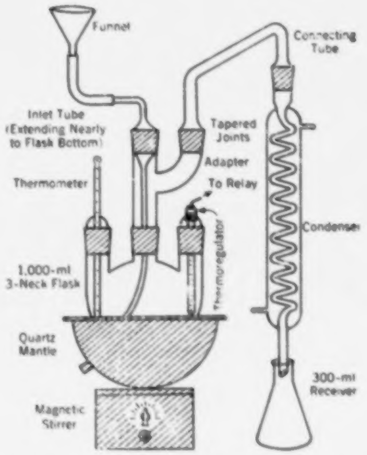

Figure 1.—*Distillation Apparatus. The setup shown provides for mixing the sample and acid, and makes possible the addition of the sample without dismantling the apparatus.*

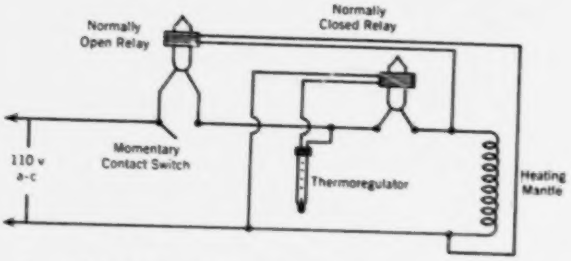

FIGURE 2.—*Control Circuit. This electrical circuit automatically stops the distillation after it is completed.*

contents reach 180° C. Add a 300-ml. sample through the inlet funnel, press the push-button switch, and the distillation will proceed without further attention. Before the distillation is begun, the condenser water must, of course, be turned on and the magnetic stirrer adjusted to provide a slow, steady mix. Subsequent distillations require only that the sample be added and the pushbutton switch depressed. If visible or audible notice of distillation completion is required, a pilot light or buzzer can very easily be added to the circuit.

RESULTS

As the apparatus and procedure remain essentially that of the original method, accuracy, precision, and precautions are essentially the same. Because of the uniform heating characteristics of the electric mantle, however, the dangers of sulfate carryover are minimized.

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Reactions of Fluoride Ion with Enamel and Dentin

There has been no final resolution of the problem as to how fluoride inhibits dental caries. Increased quantities of fluoride in dentin and enamel are proportional to fluoride ingested in drinking water and there are basic modifications in certain physical and chemical properties of enamel and dentin associated with their fluoride content. Thus there may develop a caries resistant property in those teeth which have acquired an optimum content of fluoride.

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REACTIONS OF FLUORIDE ION WITH HYDROXYAPATITE

By Harold G. McCann

A problem of long standing in the study of dentin and enamel is the exact nature and stucture of the inorganic material in these tissues. A review of these studies from the time of Berzelius in 1845 up to 1949 has been published by Armstrong *(1)*. In general the mineral component of teeth as well as bones consists chiefly of a basic calcium phosphate which is most probably hydroxyapatite *(2, 3)* Hydroxyapatites may be synthesized in the laboratory and their basic chemistry and reactions studied directly. The use of a synthetic hydroxyapatite instead of the natural hydroxyapatites, as may be found in teeth and bones, avoids possible complications introduced by extraneous chemicals present in the natural materials. Chemical methods devised to study the synthetic apatites may then be adapted for study of natural tooth substance. At the same time information is obtained concerning basic chemical reactions which take place and the effects of varying conditions on these basic reactions. In these experiments we have been concerned for obvious reasons *(4, 5)* primarily with reactions between the fluoride ion and synthetic hydroxapatite.

EXPERIMENTAL

Preparation of Hydroxyapatite—This study was made on a series of synthesized compounds ranging in empirical composition from $Ca_{9.5}(PO_4)_6(OH)_1$ having a Ca:P mole ratio of 1.593 to that corresponding to pure hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, having a Ca:P ratio of 1.667. These hydroxapatites, listed with their analyses in table I, were prepared by a modification of the acidimetric precipitation method of Rathje *(6, 7)*, later employed by Egan et al. *(8)*.

To 6 liters of continuously boiling solution (approximately 1 M in respect to an ammonium salt) in a 12 liter flask were added 2 liters each of a solution of 0.12 M KH_2PO_4 and 0.20 M $Ca(NO_3)_2$ at an equal rate of about 60 ml. per hour for 8 hour periods during the day. The slurry was left boiling overnight and addition continued the following day. Samples K and L were allowed to cool overnight and absorbed CO_2 as shown in table I. The pH was kept just alkaline to phenolphthalein, brom cresol purple, or brom phenol blue by the periodic addition of concentrated NH_4OH. The NH_4Cl first used caused the presence of 0.3 to 0.5 percent Cl as an impurity. This was avoided by the use of ammonium nitrate, but ammonium acetate or borate made it easier to maintain the desired pH. The final products were filtered on a sintered glass funnel, washed with copious quantities of distilled water, and dried at 110°. All chemicals used were reagent grade. The $Ca(NO_3)_2$ was prepared from $CaCO_3$ treated with a slight excess of HNO_3, the CO_2 was boiled off, and the excess HNO_3 neutralized.

A hydroxapatite radioactive in respect to both calcium and phosphorus was prepared from 10 gm. of $CaCO_3$ containing 1.5 mc. of Ca^{45} and 8.15 gm. of KH_2PO_4 plus 2 ml. of a solution containing 2 mc. of P^{32} as phosphate in dilute HCl. This sample was analyzed gravimetrically after it had decayed for 2½ months.

Although these preparations varied considerably in composition, it has been suggested by Bale et al. *(2)*, Hendricks and Hill *(3)*, and Posner and Stephenson *(9)* that all such materials are hydroxyapatite with entrapped acid phosphate ions on the surfaces.

Treatment of Hydroxyapatites with Fluoride.—These apatites were treated in various ways in order to determine the effect of concentration of the fluoride ion, temperature, and Ca:P ratio on reactions with the fluoride ion. Concentration of fluoride, usually as NaF, was varied from 1 part per million such as occurs naturally in water up to 2 percent NaF as used in the topical application of fluoride to the teeth. Temperatures of 20°, 37°, and 100° were employed.

Samples in the more concentrated solutions were treated by placing 2.5 gm. of solid apatite into a container and adding 100 ml. of a solution containing the desired fluoride concentration. At 37° and below, polyethylene bottles were used; at 100°, Pyrex flasks. The more dilute solutions were made up in 12 liter flasks, as a volume of 10 liters of solution was employed to obtain sufficient fluoride to react with 2.5 gm. of apatite. Smaller amounts were sometimes used when insufficient material was available; a less complete analysis was then obtained. Samples were boiled continuously to maintain 100° temperature; at lower temperatures samples were placed in a constant temperature oven or room and shaken occasionally. After a period of time varying from several days for the 100° samples to 1 to 4 months for the lower temperature samples, they were filtered, washed, and dried at 110°. The filtrates from the concentrated samples included the washings and were made up to 500 ml. One liter of filtrate from the dilute solutions was reserved.

Analytical.—The original apatites and the treated solids were analyzed gravimetrically for calcium by double precipitation of the oxalate from acid solution by slow addition of NH_4OH to avoid contamination with phosphate with final weighing as the fluoride, and for phosphate by double precipitation as magnesium ammonium phosphate to assure the proper stoichiometric relationship *(10)*. Fluoride was determined colorimetrically by visual matching or spectrophotometrically; both methods are modification of the Willard and Winter method *(11)*. In the liquid filtrates calcium was determined microvolumetrically on a concentrated aliquot by a modification of the ceric sulfate procedure of Kirk *(12)*, and phosphorus spectrophotometrically by the molybdivanadophosphoric acid procedure as modified by Simonsen et al. *(13)*

Radioactivity Measurements.—Counts were made with a thin mica window Geiger tube and a Nuclear Instrument scaling unit model No. 163. The mixed Ca^{45} and P^{32} were determined in the same

Table I.—*Analyses of hydroxyapatites prepared and used in this study*

Apatite sample No.	CaO	P_2O_5	Ratio Ca:P	Cl	CO_2	Particle size*
	per cent	*per cent*	*moles*	*per cent*	*per cent*	
I	53.32	42.40	1.593	0.35		Some particles 3 to 4 μ
K	53.54	41.62	1.628	0.51	0.31	Small; few crystals
L	53.65	41.53	1.645	0.39	0.64	Needles; some 6 to 7 μ
Q	53.65	40.83	1.663	Nil		Few very small crystals
H	53.10	40.39	1.665	"		Mostly amorphous; smaller than Sample Q
Radioactive	51.85	40.01	1.641	"		

* Microscopical observation.

sample by the use of an aluminum absorber according to the method of Comar et al. (*14*). Samples of 0.100 gm. were equilibrated with a series of 500 ml. of 1 p.p.m. to 1 percent F for 4 days at 20°; then they were filtered and the aliquots counted.

Electrolytic Conductivity Apparatus.—The Wheatstone bridge was made up of a Campbell-Shackelton shielded ratio box, Leeds and Northrup No. 1553, and a six dial shielded alternating current resistance box from 0.01 to 11,111.1 ohms, No. 4164. The 500, 1000, and 2000 cycle oscillator and amplifier were standard Leeds and Northrup instruments, Nos. 9842 and 9847. A 5 inch DuMont type 274-A oscillograph served as null-point indicator. Cell capacity was compensated by an adjustable air capacitor, No. 4914. Leeds and Northrup conductivity cells were employed and platinized according to the directions of Jones and Bollinger (*15*). During measurement, cells were immersed in a constant temperature bath held to ±0.01°. Cells were standardized with KCl solutions by using the specific conductance values and directions given by Jones and Bollinger (*16*).

The conductivity of solutions containing 1 to 1000 p.p.m. of fluoride ion as NaF mixed with hydroxyapatite sample II and equilibrated for 4 to 8 days at 37° was measured.

RESULTS

Treatment with Concentrated Fluoride Solutions to 37° (table II).—The results of treating the various hydroxyapatite samples with fluoride concentrations in and above the range used in topical application to teeth are shown in table II. By comparing the analysis of the solid before and after treatment, together with the concentration of calcium and phosphorus released into the liquid phase during reaction, it was possible to determine in what manner the fluoride had reacted with the solid: by double decomposition to form CaF_2 or by exchange with the hydroxyl to form fluorapatite. If reaction with phosphate occurs somewhat according to the reaction

$$\text{(A)}\quad Ca_{10}(PO_4)_6(OH)_2 + 20F^- \rightarrow 10CaF_2 + 6PO_4^{\equiv} + 2OH^-$$

the ratio of Ca to P in the solid will be increased, since the calcium will remain in the solid as CaF_2, while the phosphate will be removed into the solution. If this reaction occurs exclusively, the original ratio will be obtained by adjusting the experimental ratio for the calcium combined with fluoride. This is the case with samples I, K, and L, indicating the formation of calcium fluoride, and very little or no fluorapatite.

If fluorapatite forms according to the reaction

$$\text{(B)}\quad Ca_{10}(PO_4)_6(OH)_2 + 20F^- \rightarrow Ca_{10}(PO_4)_6F_2 + 2OH^-$$

or hydoxyfluorapatite according to the reaction as suggested by Giesecke

$$\text{(C)}\quad Ca_{10}(PO_4)_6(OH)_2 + F^- \rightarrow Ca_{10}(PO_4)_6FOH + OH^-$$

and Rathje (*17*), the Ca:P ratio of the solid remains unchanged, as the hydroxyl group is the only portion of the molecule affected. If the ratio is adjusted to calcium equivalent to fluoride in this case, the ratio will be lower than that in the original apatite. Samples Q and II give results indicating considerable fluorapatite formation at fluoride concentrations up to 0.2 percent, and probably some fluorapatite along with the CaF_2 at higher fluoride concentrations with sample II.

The calcium and phosphate content of the liquid phase also indicates in most cases a similar type of reaction. Since the calcium content is low, it was assumed that if calcium fluoride were formed most of the phosphate in solution would be due to replacement by fluoride. According to reaction A above, 20 fluoride ions replace 6 phosphate ions so that moles of P × 3.33 = moles of F. This gives results which are somewhat high since some of the phosphate is due to the apatite solubility. However, if moles of P are multiplied by 3, results are obtained which indicate somewhat quantitatively the amount of phosphate replaced, although, when the amount of fluoride which reacted is low, a greater proportion of the phosphate is due to apatite solubility.

Treatment with Concentrated Fluoride Solutions at 100° (table III).—By a similar calculation it is seen that sample I has reacted mostly according to reaction A as before, but sample L has reacted to form fluorapatite according to reaction B, while sample K, although forming mostly CaF_2, has reacted partially as in reaction B.

Dilute and Intermediate Values of Fluoride Concentrations at 37° (tables

TABLE II.—*Treatment of hydroxyapatites with concentrated fluoride solutions (2.5 gm. of hydroxyapatite treated at 37° with 100 ml. of the indicated sodium fluoride solution)*

Apatite sample No.	Original solution	Solid					Filtrate			Solid
	F	CaO	P_2O_5	F	Ca:P ratio	Adjusted Ca:P‡	P	Ca	P§	F
	per cent	*per cent*	*per cent*	*per cent*	*moles*	*moles*	*μM*	*μM*	*μM × 3*	*μM*
I (1.593)*	0.09	53.56	42.45	0.10	1.598	1.593	41	22	123	131
	0.95	53.60	41.89	0.52	1.620	1.596	250	9	750	690
K (1.628)	0.09	53.70	41.48	0.21	1.639	1.629	94	27	282	270
L (1.645)	0.095	53.77	41.24	0.04	1.651	1.648	16	22	48	46
	0.95	54.01	41.08	0.38	1.665	1.647	173	22	519	500
Q (1.663)	0.095	53.89	41.05	0.07	1.662	1.659	20	14	60	92
	0.19	53.96	41.08	0.09	1.663	1.659	27	14	81	118
	0.475	54.39	40.77	0.39	1.689	1.671	167	23	501	510
	0.950	54.60	40.47	0.74	1.708	1.674	323	19	969	970
II (1.665)	0.095	52.96	40.31	0.19	1.663	1.655	40	28	120	250
	0.19	52.89	40.19	0.24	1.666	1.655	48	28	144	320
	0.95	53.24	39.89	0.80	1.690	1.652	290	42	870	1,050
	1.90	53.36	38.84	1.74	1.739	1.656	833	5	2,499	2,290
II (KF)†	0.095	53.10	40.51	0.21	1.660	1.650	51	13	153	280
	9.5	57.08	25.49	17.4	2.835	1.56	5980	Low	17,940	22,900
II (NH_4F)†	0.095	53.20	40.43	0.52	1.666	1.642	142	9	426	680

* The ratio of the original apatite is given in parentheses for convenience in referring to changes occurring through reaction with fluoride.

† KF and NH_4F substituted for NaF used in previous samples. The KF permits a higher fluoride concentration due to much greater solubility. The increased reaction with the NH_4F is due to its greater acidity.

‡ Actual Ca:P ratio adjusted by subtracting the CaO equivalent of the F from the actual per cent CaO.

§ Actual moles of phosphorus × 3, as approximately 1 mole of P would be released for each 3 moles of F in a direct replacement reaction. This calculation allows a ready comparison with the amount of fluoride reacted.

Table III.—*Treatment with concentrated fluoride solutions (2.5 gm. of hydroxyapatite treated at 100° with 100 ml. of the indicated sodium fluoride concentration)*

Apatite sample No.	Original solution	Solid					Filtrate			Solid
	F	CaO	P_2O_5	F	Ca:P ratio	Adjusted Ca:P‡	P	Ca	P§	F
	per cent	*per cent*	*per cent*	*per cent*	*moles*	*moles*	*μM*	*μM*	*μM × 3*	*μM*
I (1.593)*	0.09	53.27	42.07	0.17	1.603	1.594	55	11	165	223
	0.18	53.22	42.02	0.18	1.604	1.595	54	10	162	236
	0.91	53.57	41.15	1.29	1.648	1.589	550	20	1650	1690
K (1.628)	0.09	53.20	41.06	0.31	1.641	1.626	113	11	340	408
	0.18	53.33	41.14	0.39	1.641	1.623	113	16	340	510
L (1.645)	0.18	53.58	41.33	0.19	1.641	1.632	49	11	147	250
Q (1.663)	0.095	53.90	41.03	0.16	1.663	1.656	33	8	99	210
	0.95	52.89	38.24	1.81	1.751	1.662	740	13	2220	2380

See foot-notes in Table II.

IV and V).—The results of treating the hydroxyapatite samples with the fluoride concentrations found in natural waters and with intermediate values up to those used in topical application to teeth are shown in table IV. The first four values of the Ca:P ratio in table IV are somewhat lower than the original hydroxyapatite due to reaction with the large volume of water. As the fluoride concentration goes up, this effect is overcome. However, since the formation of calcium fluoride would increase this ratio considerably, the assumption that fluorapatite has formed is believed to be valid. Up through 100 p.p.m. of fluoride, only fluorapatite is formed; above this, considerable amounts of CaF_2 are formed. Although the fluoride concentration in the 1000 p.p.m. solution is about the same as that in the 0.095 percent solution in table II, where mostly fluorapatite was formed, the formation of CaF_2 in this case (table IV) may possibly be accounted for by the 100-fold difference in the ratio of solid to fluoride ion.

At this dilution considerably more apatite dissolves compared to the amount of fluoride which reacted, so that the phosphate is not considered in excess unless the concentration is greater than the calcium concentration. Thus the liquid phase analyses indicate the occurrence of the same reactions as those indicated by the solid Ca:P ratios. In the first half of table V are given liquid phase values of some of the apatites in which insufficient material was available for complete solid analyses. Apparently at this concentration fluorapatite has formed in all cases.

Dilute and Intermediate Values of Fluoride Concentrations at 100° (tables V and VI).—Up to 20 p.p.m. of fluoride, apatite sample II forms only fluorapatite, but, starting at about 100 p.p.m., ever increasing amounts of CaF_2 are formed. At low fluoride levels apatite samples L and Q also form fluorapatite, but apatite samples I and K release excess phosphate. Since CaF_2 cannot form at this fluoride concentration, it is possible that adsorbed phosphate is being released at this temperature. However, the probability is that these samples are not stable at this temperature and dilution and are becoming more basic in the boiling water, thus releasing phosphate while at the same time forming some fluorapatite.

Radioactive Hydroxyapatite.—This material with a calcium to phosphorus ratio of 1.641 is similar in this respect to apatite sample L with a ratio of 1.645. The reactions with this material took place at a somewhat lower temperature (20°) and for a considerably shorter time than with other samples, so that the attainment of equilibrium is less likely. As shown in figure 1, there is a drop in both the calcium and phosphorus concentrations at first, showing the decreased solubility of fluorapatite as compared with hydroxyapatite. There is no indication of reaction to form CaF_2 below 100 p.p.m., but at 1000 p.p.m. this type of reaction was extensive as indicated by the higher P^{32} concentration in the filtrate. Apatite sample L showed a similar reaction at this low concentration at 37°. It is interesting to note that there is no sudden change in the type of reaction as the solubility product concentration (about 25 p.p.m. of F, based on the solubility of the apatite in water) is passed, but that a change occurs gradually as a level considerably above this value is approached.

Electrolytic Conductivity.—It is interesting to note again that there is no indication of any change in the type of reaction as the solubility product concentration is passed. A change in the type of reaction at this point would probably change the slope of the curve somewhat, due to a difference in the ions released. However, a perfectly straight line was obtained up to 50 p.p.m., as shown in figure 2.

Effect of Buffering Solutions (table VII).—The lower the pH, the greater the solubility of the hydroxyapatite and hence the greater the amount of CaF_2 formed from the dissolved calcium. After

Table IV.—*Dilute and intermediate values of fluoride concentrations (2.5 gm of hydroxyapatite treated at 37° with 10 liters of the indicated sodium fluoride concentration)*

Apatite sample No.	Original solution	Solid					Liquid*			Solid*
	F	CaO	P_2O_5	F	Ca:P	Adjusted Ca:P†	P	Ca	Excess P‡	F
	γ per ml.	*per cent*	*per cent*	*per cent*	*moles*	*moles*	*μM*	*μM*	*μM*	*μM*
Q (1.663)	2	53.84	41.18	0.05	1.655	1.653	29	30	0	7
II (1.665)	2	52.97	40.50	0.20	1.656	1.646	27	55	0	26
	5	53.08	40.60	0.22	1.655	1.646	16	28	0	29
	10	53.13	40.61	0.32	1.656	1.642	16	22	0	42
	20	53.26	40.52	0.32	1.663	1.650	26	36	0	42
	50	53.11	40.58	0.37	1.657	1.639	22	30	0	49
	100	53.15	40.49	0.31	1.662	1.648	35	39	0	41
	200	53.27	40.24	0.66	1.676	1.645	48	8	40	87
	500	53.40	39.71	1.32	1.702	1.640	123	3	120	174
	1000	53.70	38.56	2.43	1.763	1.645	145	2	140	320

* Concentrations are expressed in micromoles per liter in the liquid and micromoles per 0.25 gm. of the solid, so that direct comparison may be made.

† As in Table II.

‡ At this dilution, 100 times that of previous samples, the solubility of the apatite in relation to the amount of fluoride which reacted is considerably higher and phosphate is considered to be in excess only if its concentration exceeds that of the calcium.

Table V.—*Dilute values of fluoride concentrations (0.10 gm. of hydroxyapatite treated with 400 ml. of solution containing 5 p.p.m. of fluoride at the indicated temperature)*

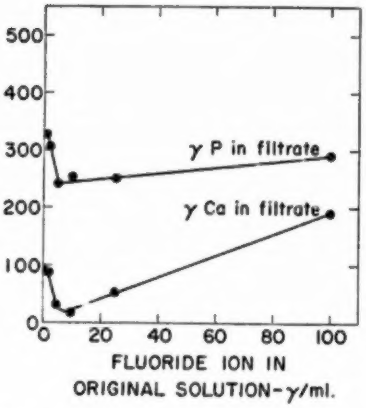
Apatite sample No.	Temperature	Solid	Liquid*			Solid*
		F	P	Ca	Excess P‡	F
	°C.	*per cent*	*μM*	*μM*	*μM*	*μM*
I	37	0.06	32	42	0	8
K		0.20	29	29	0	26
L		0.01	29	37	0	1
II		0.28	29	24	0	37
I	100	0.20	103	55	40	26
K		0.2	30	8	22	26
L		0.1	10	22	0	13
Q		0.17	10	18	0	43

* See foot-notes in Table IV.

Table VI.—*Dilute and intermediate values of fluoride concentrations (2.5 gm. of hydroxyapatite treated at 100° with 10 liters of the indicated sodium fluoride concentration)*

Apatite sample No.	Original solution	Solid					Liquid*			Solid*
	F	CaO	P_2O_5	F	Ca:P ratio	Adjusted Ca:P†	P	Ca	Excess P‡	F
	γ per ml.	*per cent*	*per cent*	*per cent*	*moles*	*moles*	*μM*	*μM*	*μM*	*μM*
I (1.593)	10	52.30	41.51	0.12	1.609	1.599	Lost	27	0	16
II (1.665)	10	52.20	40.00	0.33	1.653	1.637	16	29	0	43
	20	53.21	40.48	0.38	1.663	1.647	20	25	0	50
	100	51.19	38.93	0.58	1.672	1.643	84	7	77	76
	200	53.02	40.10	0.47	1.674	1.652	129	4	125	62
	500	52.41	39.69	0.67	1.672	1.640	145	2	140	88
	1000	53.11	38.40	2.18	1.751	1.641	1340	6	1330	287

* See foot-notes in Table IV.



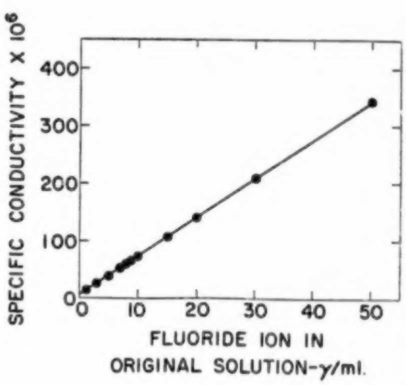


Figure 1.—*The effect of F ion on radioactive hydroxyapatite, showing the concentration of Ca and P in the filtrates from 0.1 gm. samples of apatite treated at 20° with 500 ml. of the indicated F solutions.*

Figure 2.—*Electrolytic conductivity of solutions of apatite sample II and F ion.*

the acidity has been neutralized by dissolving the hydroxyapatite, apparently considerable fluorapatite also forms.

DISCUSSION

These experiments show that the most important variables in the reaction between fluoride ion and hydroxyapatite are the fluoride concentration and the Ca:P mole ratio of the hydroxyapatite, with the temperature playing a less important role. At 37° at a concentration of 5 p.p.m. of fluoride, some fluorapatite is formed with all the samples of hydroxyapatite tried. With 100 percent hydroxyapatite, fluorapatite is formed almost exclusively up to 0.1 to 0.2 percent fluoride when small volumes of solution are used (2.5 gm. per 100 ml. of solution), and up to 200 p.p.m. with large volumes of solution (2.5 gm. per 10,000 ml. of solution) by exchange of fluoride with hydroxyl. At concentrations of around 0.1 or 0.2 percent fluoride at 37°, little or no fluorapatite is formed until the ratio of calcium to phosphorus is over three-fourths of the way between tricalcium phosphate (1.500) and hydroxyapatite (1.667). At 100° the formation of fluorapatite starts just a little over half way between the two, as indicated in figure 3.

The formation of fluorapatite from hydroxyapatite (as contained in fertilizer or bone) at fluoride levels in which the solubility product has not been exceeded has been shown by several workers. MacIntire et al. (*18*) found this to be the cause of the insolubility developed by certain phosphatic fertilizer mixtures on storage. Neuman et al. (*19*) demonstrated that fluoride replaces either hydroxyl or bicarbonate ions in the surfaces of the mineral phase of bone. Megirian (*20*) confirmed these results with glycolashed bone.

All these workers used very low fluoride concentrations and the evidence for fluorapatite formation at higher concentrations has been very sparse. Reference has often been made to the importance of keeping the fluoride concentration at a level in which the solubility product is not exceeded (*19, 20*). Syrrist (*21*), in a histological study of teeth exposed to a strong NaF solution for 20 minutes in vivo, suggested the presence of fluorapatite rather than calcium fluoride after extraction 1 week later. Variability of reaction with fluoride with varying Ca:P ratio has been indicated by MacIntire et al. (*22*), who noted that a number of commercially prepared calcium phosphates reacted in varying degree with CaF_2 to form fluorapatite, pure hydroxyapatite being the most reactive.

Table VII.—*Effect of buffering solutions (2.5 gm. of hydroxyapatite sample II in buffer at indicated pH; 0.09 percent F at 37°)*

Glycine buffer		Solid					Filtrate			Solid
Original pH	Final pH	CaO	P_2O_5	F	Ca:P ratio	Adjusted Ca:P‡	P	Ca	P§	F
		per cent	*per cent*	*per cent*	*moles*	*moles*	*μM*	*μM*	*μM × 3*	*μM*
2.95	7.40	52.99	39.56	1.36	1.696	1.631	342	12	1026	1790
3.93	7.38	52.85	39.90	0.71	1.677	1.644	197	6	591	930
5.97	6.90	53.19	40.33	0.39	1.670	1.651	84	12	252	510
7.97	6.99	53.20	40.39	0.36	1.667	1.651	81	12	243	470
8.95	8.54	53.04	40.47	0.25	1.659	1.648	65	12	195	330
Phosphate buffer										
5.07	6.33	53.44	40.58	1.16	1.667	1.614				1530
6.10	6.57	53.20	40.88	0.79	1.647	1.611				1040
6.98	7.23	53.18	40.99	0.61	1.643	1.615				800
8.00	8.33	53.04	40.80	0.27	1.646	1.633				360

See foot-notes in Table II.

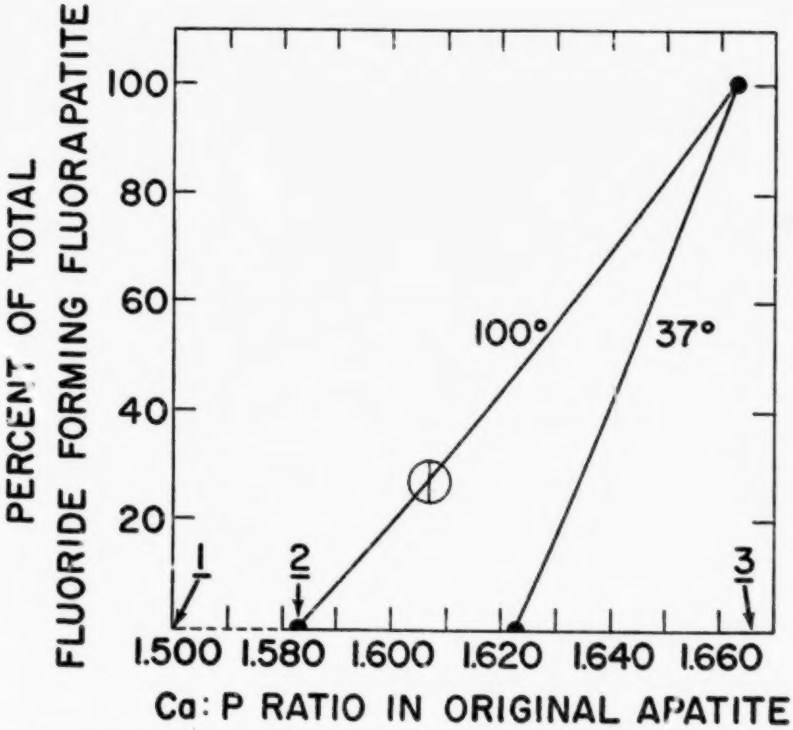


Figure 3.—*Effect of Ca:P ratio of apatite on the type of reaction with 0.1 to 0.2 percent F. The ratio at point 1 is that of tricalcium phosphate, at point 3 that of hydroxyapatite, and point 2 is midway between, a point at which the empirical composition is $Ca_{9.5}(PO_4)_6(OH)_1$. The points on the 100° line represent reactions with apatite samples I, K, and Q, and on the 37° line with apatite samples L and Q or II, as estimated from the data in tables II and III. Ca:P ratios have been adjusted for Cl and CO_3 content.*

The reaction of fluoride concentrations above 0.2 percent has been shown in the above experiments to form calcium fluoride in large amounts with small amounts of fluorapatite also forming in certain cases with 100 percent hydroxyapatite. This formation of calcium fluoride has been shown by several workers to be due to the double decomposition of calcium phosphate by sodium fluoride to form calcium fluoride (*23–26*).

This reaction appears to take place on the surface of the crystals. Unless the fluoride concentration is very high, forcing a considerable breakdown of the hydroxyapatite structure, the amount of reaction as indicated by the fluoride content of the solid changes but slightly with fluoride concentration in the solution. Thus the per cent of fluoride in apatite sample II ranges from 0.2 percent to 0.3 percent as the fluoride concentration increases from 2 to 100 p.p.m. There is more of a change in the amount of fluoride picked up by apatites with similar Ca:P ratios but different surface areas than there is among apatites with widely different Ca:P ratios and similar surface areas. However, the type of reaction which takes place is dependent to a great degree on the hydroxy concentration on the surfaces.

SUMMARY

The reactions between fluoride ion and hydroxyapatite were investigated over a fluoride range of 1 p.p.m to 9.5 percent, and over a calcium to phosphorus ratio in the apatites of 1.593 to 1.665, with results indicating that the type of reaction is chiefly dependent on these two variables. Fluorapatite is formed at all ratios of Ca:P at a few parts per million of fluoride, calcium fluoride or fluorapatite is formed up to 0.2 percent fluoride, depending on the Ca:P ratio, and calcium fluoride is formed at all ratios at high fluoride concentrations.

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REACTIONS OF FLUORIDE ION WITH POWDERED ENAMEL AND DENTIN

HAROLD G. McCANN and FREDERICK A. BULLOCK

A report on reactions of the fluoride ion with synthetic hydroxyapatite was presented recently (*1*). It was shown that with relatively pure hydroxyapatite there is a gradual change in the type of reaction as the fluoride concentration increases. With a high ratio of liquid phase to solid (10 l. of solution to 2.5 gm. of apatite), fluorapatite is formed at the lowest concentrations of F^- and gradually, as the fluoride concentration increases beyond 100 p.p.m., more and more of the fluoride is converted to calcium fluoride. With a low ratio of liquid to solid (100 ml. of solution and 2.5 gm. of apatite), the formation of fluorapatite is the chief reaction up to a concentration of 0.2 per cent fluoride; beyond this point, more and more calcium fluoride is formed.

Several mechanisms for the reaction of fluoride with enamel, dentin, and bone have been suggested. Neuman, Neuman, Main, O'Leary, and Smith (*2*) have shown that fluorapatite is formed below the solubility product of calcium fluoride. Falkenheim and Hodge (*3*), and Volker, Hodge, Wilson, and Van Voorhis (*4*) have shown that surface adsorption of fluoride takes place. The double decomposition of apatite with high levels of fluoride has been demonstrated by Gerould (*5*), Scott, Picard, and Wyckoff (*6*), and others. This paper presents the results of the extension of the work on synthetic hydroxyapatite to powdered enamel and dentin and an attempt to correlate these findings with those of other workers.

EXPERIMENTAL

Preparation of Enamel and Dentin Samples.—Several thousand human teeth were cleaned of fillings and decayed material with a dental burr, crushed in a steel tube, ground to pass a 60 mesh sieve, and fat extracted with alcohol and ether. Enamel and dentin were then separated by the procedure of Manly and Hodge (*7*). Each sample was then separated into 12 sieve fractions, and surface areas from 4 fractions each of enamel and dentin were obtained. The surface areas and chemical analyses are shown in table I. For most of the work, a mixed sample made up from a number of these screened samples (excluding -325 fine) was used.

Treatment of Enamel and Dentin With Fluoride.—Samples of both enamel and dentin were treated in two series. In one, 2.5 gm. of solid was added to 10 l. of solutions ranging from 2 to 2,000 p.p.m. fluoride as NaF. In the other, 2.5 gm. samples were treated with 200 ml. of solution ranging from 0.05 to 2 percent fluoride as NaF. These concentrations were used to simulate the fluoride concentrations which may occur in natural waters and those used in the topical application of fluoride to the teeth. The first series, made up in 12 l. flasks, and

TABLE I.—*Analytical data on enamel and dentin samples*

SAMPLE	AREA (M² PER GM.)	CaO (%)	P_2O_5 (%)	Ca:P (MOLES)	MgO (%)	CO_2 (%)	Na (%)
Mixed enamel	—	[illegible].89	40.02	1.610	0.53	3.01	0.80
60-80 Mesh	0.94	51.27	40.15	1.616	0.53	2.89	—
100-120 Mesh	1.2	51.13	40.06	1.616	0.47	—	—
200-230 Mesh	1.5	50.90	40.29	1.600	0.39	—	—
-325 (Fine)	9.7	49.11	38.53	1.614	0.57	—	—
Mixed dentin	—	37.29	29.56	1.597	1.31	3.21	0.71
60-70 Mesh	3.2	37.08	29.28	1.603	1.22	3.22	—
100-120 Mesh	3.9	37.17	29.45	1.598	1.21	—	—
200-230 Mesh	4.0	37.48	29.87	1.590	1.30	—	—
-325 (Fine)	11.2	44.82	35.24	1.610	0.84	3.00	—

the second, made up in 250 ml. polyethylene bottles, were placed in an incubator room at 37° C. and shaken daily over a period of 3 to 4 weeks, then filtered, and dried at 110° C. The filtrates from the concentrated solutions included the washings and were made up to 1 l. One liter of filtrate from each of the dilute solutions was reserved for analysis.

Electrolytic Conductivity.—The conductivity of a separate series of solutions (0.1 gm. solid in 250 ml. H_2O) containing 1 to 1,000 p.p.m. of fluoride ion as NaF, mixed with enamel in one series and dentin in another series and equilibrated for 1 to 2 weeks at 37° C., was measured in the apparatus previously described (*1*).

Analytical.—Samples were analyzed for calcium, phosphorus, and fluoride as described previously (*1*), except that a triple precipitation of calcium was employed to ensure a more complete separation of the magnesium. In the solid samples, magnesium was determined in the filtrate of the calcium determination by double precipitation as magnesium ammonium phosphate, as described by Kolthoff and Sandell (*8*). Magnesium was determined in the filtered solutions by the thiazole yellow method, as modified by Young and Gill (*10*). Carbon dioxide was determined either by evolution with $HClO_4$ and absorption of the dried gas in a weighing bulb, a modification of the standard procedure as given by Kolthoff and Sandell (*8*), or by measurement of the volume of evolved gas in a Warburg apparatus. Good agreement was obtained by the two methods. Sodium was determined in the Beckman Model DU Flame Spectrophotometer (*9*). Interference of calcium and phosphorus was overcome by adding similar amounts to the sodium standards.

RESULTS

Treatment of Enamel and Dentin With Dilute and Intermediate Fluoride Solutions (table II).—The methods of calculation previously used (*1*) with hydroxyapatite are not completely applicable here because of hydrolysis of the samples. The inorganic portion of enamel and dentin consists of hydroxyapatite with varying Ca:P ratios caused by the adsorption of acid phosphate ions on crystal surfaces along with other ions including carbonate, citrate, sodium, and magnesium (*11*). In dilute solutions, some of these ions are removed from the surfaces with resultant change in the composition of the solid. Thus, as shown in table II, the Ca:P ratio at 2 p.p.m. fluoride has increased from 1.610 to 1.627 in the enamel and from 1.597 to 1.642 in the dentin. Since at this concentration the increase cannot be due to the formation of insoluble calcium fluoride, it is most probably caused by the removal of some of the adsorbed acid phosphate ions into solution. This is also indicated by the calcium and phosphate content of the filtrates. Also, about 30 percent of the magnesium has been leached from the dentin (1.3 percent to 0.9 percent) and somewhat less from the enamel (see tables I and II).

Table II.—*Treatment of enamel and dentin with dilute and intermediate fluoride solutions* *

SAMPLE	ORIGINAL SOLUTION F (γ/ML.)	SOLID CaO (%)	SOLID P_2O_5 (%)	SOLID Ca:P MOLES	SOLID F (%)	SOLID MgO (%)	SOLID Na (%)	SOLID CO_2 (%)	LIQUID P (μM)	LIQUID Ca (μM)	LIQUID Mg. (MG.)
	0	—	—	—	—	—	—	2.78	260	530	
Mixed enamel	2	51.47	40.04	1.627	0.09	0.48	0.71	—	260	350	
(1.610)†	7	51.59	40.07	1.630	0.12	0.42	0.71	2.62	260	290	
	10	51.41	39.90	1.631	0.15	0.46	—	—	260	310	
	25	51.49	40.03	1.628	0.21	0.48	—	—	270	270	
	50	51.85	40.25	1.631	0.28	0.47	—	—	210	210	
	100	51.52	39.85	1.637	0.28	0.48	—	—	370	290	
	500x	51.33	39.57	1.642	0.47	0.46	0.77	—	275	30	
	1,000x	51.31	39.35	1.651	0.77	0.53	—	2.66	530	25	
	2,000x	51.53	38.58	1.691	1.58	0.55	0.90	—	810	60	
	0	—	—	—	—	—	—	3.08	578	770	8.2
Mixed dentin	2	40.16	30.96	1.642	0.56	0.92	0.39	2.75	450	430	7.9
(1.597)†	7	40.04	31.06	1.632	0.86	0.95	0.42	—	450	310	6.8
	10	39.75	30.88	1.630	0.99	0.95	—	—	390	250	6.3
	25	40.08	31.09	1.632	1.25	0.95	—	—	450	290	5.4
	50	40.10	30.87	1.645	1.44	1.07	0.43	—	480	180	4.7
	100	39.92	30.35	1.665	1.55	1.09	0.46	—	520	180	4.6
	500x	40.45	30.39	1.685	1.96	1.29	0.70	—	550	45	0.5
	1,000x	40.15	29.91	1.699	2.22	1.35	0.77	2.51	565	43	—
	2,000x	40.28	29.72	1.716	4.40	1.27	1.93	2.52	970	—	—

*Two and one-half grams of enamel or dentin treated with 10 L. of solution, except those marked "x," which were treated with 5 L.

†Ca:P ratio of original material.

The Ca:P ratios of both the enamel and dentin change but slightly on treatment with solutions containing from 2 p.p.m. through 50 p.p.m. fluoride. The results of analyses also show that the solubility of the solid (as shown by the dissolution of calcium and phosphorus) decreases slightly over this range and considerably between 0 and 2 p.p.m. because of the formation of fluorapatite, which is less soluble than hydroxyapatite.

Another reaction which might take place which would not change the Ca:P ratio is the adsorption of fluoride on the crystal surfaces by exchange with CO_2, probably as bicarbonate ion. This exchange is suggested by the decrease in CO_2 content when fluoride content increases, although the evidence is insufficient for definite conclusions to be drawn. Other negative ions, such as citrate, may also be exchanged. Another possibility for uptake of fluoride is by direct adsorption. In order to keep the crystals electrically neutral, a positively charged ion must be adsorbed with the negatively charged F^-. In the case of NaF, this cation would be Na^+. Thus a measure of the Na content in the solid should give some idea of the F^- uptake by direct adsorption. These results are also shown in table II. The adsorption on enamel surfaces is slight over most of the range, but is considerable on the dentin, and a large portion of the F^- is apparently adsorbed directly at 2,000 p.p.m., as the F^- cannot be accounted for by other mechanisms. The possibility that the organic matrix is involved in the adsorption has not been eliminated. An analysis for sodium was made on hydroxyapatite sample II treated with fluoride ion over the range 2 to 1,000 p.p.m. as described previously (*1*). No adsorption at all took place except at 1,000 p.p.m., where a very slight amount occurred (0.08 percent).

In the case of the dentin, and to a limited extent of the enamel, another reaction occurs. Calculations of the solubility product of MgF_2 show that as the fluoride concentration goes up, the solubility product is exceeded, and more and more of the magnesium leached from the solid is reprecipitated as MgF_2 and filtered off with the solid.

At 500 p.p.m. and above, considerable amounts of calcium fluoride are formed by double decomposition. In this range the reactions of enamel and dentin are similar in respect to CaF_2 formation. This is indicated by the considerable increase in the Ca:P ratio and also by the increase in the phosphate content of the filtrates. The additional increase in fluoride content of the dentin over the enamel at 2,000 p.p.m. is due to adsorption, as indicated by the high sodium adsorption.

TABLE IV.—*Effect of surface area on reaction with fluoride**

SAMPLE	SOLID CaO (%)	P$_2$O$_5$ (%)	F (%)	MgO (%)	Ca:P MOLES	ORIGINAL Ca:P	LIQUID P (μM)	Ca (μM)	EXCESS P (μM)	SOLID F (μM)
Enamel:										
0.94 M²/Gm.	51.36	40.15	0.09	0.50	1.619	1.616	190	230	0	113
1.2 M²/Gm.	—	40.44	0.10	0.47	—	1.616	230	290	0	125
1.5 M²/Gm.	51.47	40.20	0.12	—	1.621	1.600	190	250	0	152
9.7 M²/Gm.	50.43	39.03	0.39	—	1.636	1.614	320	305	0	502
Dentin:										
3.2 M²/Gm.	39.68	30.80	0.69	1.17	1.628	1.603	360	290	0	907
3.9 M²/Gm.	39.99	30.88	0.91	1.09	1.639	1.598	450	—	0	1,200
4.0 M²/Gm.	40.70	31.15	1.07	0.94	1.650	1.590	550	410	0	1,410
11.2 M²/Gm.	48.14	37.06	0.83	0.63	1.645	1.610	480	420	0	1,090

*Two and one-half grams of sample treated with 10 L. of solution containing 7 ppm fluoride.

Treatment of Enamel and Dentin With Concentrated Fluoride Solutions (table III).—In the interpretation of these figures, as with those in table II, the methods used in the calculations with synthetic hydroxyapatite (*1*) are not entirely applicable. Calculations of the Ca:P ratio of the first few enamel samples corrected for the presence of CaF$_2$, as was done with hydroxyapatite, gives approximately the original ratio of 1.610. This would indicate approximately 100 percent CaF$_2$ formation. However, the phosphate released is not sufficient to account for all the fluoride in the solid, particularly at 0.19 percent F$^-$. If the phosphate content in moles is multiplied by 3, or even 3.3, to give moles of phosphate equivalent to moles of fluoride (assuming the formation of CaF$_2$ by double decomposition), only about one-half the fluoride is accounted for. Since the samples subjected to lower fluoride concentrations would be even less likely to form CaF$_2$ exclusively, it is believed that solution of adsorbed phosphate has occurred to some extent and that a part of the fluoride has formed fluorapatite, part adsorbed on the crystal surfaces directly, and possibly part exchanged with CO$_2$ on the surfaces. At higher concentrations, particularly at 1.9 and 2.0 percent, although some F$^-$ adsorption has occurred in the enamel (as indicated by the sodium content), the major portion of the fluoride has formed CaF$_2$. In the dentin, although CaF$_2$ has similarly formed, considerable fluoride adsorption has also occurred.

Effect of Surface Area (table IV).—The reactivity of enamel and dentin as indicated by F$^-$ uptake increases with increase in surface area, although not in direct proportion. This is also shown by the increased amount of hydrolysis with decreasing particle size. The most finely divided dentin sample does not follow the expected series. Its analysis, however, seems to resemble enamel more than dentin, and apparently these finest particles failed to separate effectively in the Manly and Hodge procedure. This is also the case with the enamel but to a lesser extent. The dentin particles are much more reactive than the enamel particles, which cannot be accounted for solely on the basis of surface area. That the inner enamel surfaces are less accessible than those of bone has been pointed out previously by Hendricks and Hill (*11*). Dentin would be expected to be somewhat similar to bone in this respect.

Electrolytic Conductivity. — Conductivity measurements indicate, as with synthetic hydroxyapatite (*1*), that there is no sudden change in the type of reaction with fluoride as concentration increases. A plot of the results consists of straight lines slightly displaced. The enamel curve is above the synthetic apatite, and the dentin is above the enamel as each releases more ions to the solution by hydrolysis or desorption.

DISCUSSION

The reactions of enamel, and particularly of dentin, have been shown to be much more complicated than those of synthetic hydroxyapatite (*1*) in the presence of fluoride ions. Thus the hydroxyapatite appears to form fluorapatite at low fluoride concentrations and then gradually more and more calcium fluoride as the concentration increases. Flouride ion, however, reacts with dentin or ions eluded or dissolved from dentin in several different ways; namely, (a) possible exchange with CO$_2$ on the crystal surfaces, (b) direct adsorption, (c) precipitation as magnesium fluoride of the magnesium released from crystal surfaces, (d) double decomposition at high fluoride levels to form CaF$_2$ and Na$_2$HPO$_4$, and (e) formation of fluorapatite by exchange with hydroxyl ion. Enamel reacts similarly, but to a much more limited extent.

Assuming that the previously mentioned mechanisms explain the ways in which fluoride ion reacts with powdered enamel and dentin, it is possible, from the results in table II and table III, to calculate the approximate amount of fluoride in the solid contributed by each of these reactions.

(a) *Exchange With CO$_2$.*—Exchange with carbonate may take place to a limited extent as indicated by the decrease in CO$_2$ content. If it is assumed that the CO$_2$ is present as bicarbonate, as indicated by Neuman, Neuman, Main, O'Leary, and Smith (*2*), each molecule of CO$_2$ lost from the solid would be equivalent to one of fluoride gained. In the dentin this would amount to about 0.1 percent F$^-$ at 2 p.p.m. and 0.25 percent at 2,000 p.p.m. F.$^-$ In the enamel this exchange probably occurs to a maximum of 0.1 percent F$^-$ at 2,000 p.p.m.

(b) *Adsorption.*—If sodium adsorption may be used as an indication of fluoride adsorption, a mole for mole calculation may be made. In the dentin this would amount to about 0.01 percent F at 2 p.p.m. and 1.3 percent at 2,000 p.p.m. In the enamel a maximum of about 0.2 percent is reached at 2,000 p.p.m.

(c) *Magnesium Fluoride Precipitation.*—The elution in distilled water of 8 mg. of magnesium from the 2.5 gm. of dentin,

TABLE III.—*Treatment of enamel and dentin with concentrated fluoride solutions**

SAMPLE	ORIGINAL SOLUTION F (%)	SOLID CaO (%)	P$_2$O$_5$ (%)	CA:P MOLES	F (%)	Na (%)	CO$_2$ (%)	LIQUID P (μM)	Ca (μM)	P (μMx3)	SOLID F (μM)
Mixed enamel (1.610)†	0.048	51.17	39.84	1.626	0.30	0.85	—	103	34	309	394
	0.095	51.05	39.73	1.627	0.30	—	2.75	100	17	300	394
	0.190	51.13	39.76	1.628	0.38	—	—	86	8	258	500
	0.475	51.27	39.55	1.641	0.50	—	—	213	11	639	658
	0.950	—	—	—	—	—	2.58	370	13	1,010	2,660
	1.900	51.01	38.37	1.683	2.04	1.07	—	810	25	2,430	2,680
	2.000x	52.12	38.49	1.714	2.26	—	—	1,162	4	3,486	2,970
	0.000	—	—	—	—	—	3.20	—	—	—	—
Mixed dentin (1.597)†	0.048	39.28	29.99	1.658	1.40	—	2.76	307	—	921	1,840
	0.095	39.87	30.24	1.669	1.56	0.75	—	345	7	1,035	2,050
	0.190	40.25	30.63	1.664	1.60	0.81	—	339	2	1,017	2,100
	0.475	40.32	30.47	1.675	2.00	1.01	—	400	3	1,200	2,630
	0.950	40.31	29.71	1.718	3.12	1.40	2.56	620	3	1,860	4,100
	1.900	41.09	28.55	1.822	4.60	—	2.55	1,357	7	4,071	6,050
	2.000x	41.16	28.91	1.802	4.40	0.96	—	1,065	5	3,195	5,790

*Two and one-half grams of enamel or dentin treated with 200 ml. of solution, except those marked "x," which were treated with 500 ml.

†As in Table II.

amounting to about 25 percent of the total magnesium content, is additional proof that dentin, as indicated by Hendricks in the case of enamel and bone (*11*), varies in composition due to adsorbed ions on the surfaces. The Mg is desorbed in a dilute aqueous solution, and then, as the fluoride content of the solution increases, the solubility product of MgF_2 is exceeded, and thus more and more Mg is precipitated as MgF_2. This is filtered off with the solid and included in the fluoride content of the solid. The difference between the magnesium content of the filtrate from the water-treated dentin sample and a fluoride-treated dentin sample gives the amount of Mg carried down with the solid by fluoride. Assuming this to be MgF_2, the fluoride content may be calculated. In the dentin this amounts to 0.02 percent F^- at 2 p.p.m. fluoride and 0.5 percent F^- at 2,000 p.p.m. In enamel a maximum of 0.1 percent is reached.

(d) *Calcium Fluoride Precipitation.*—Calcium fluoride is formed by double decomposition of NaF and $Ca_{10}(PO_4)_6(OH)_2$ when the fluoride content of the treating solution becomes sufficiently great. This is indicated by a rise in Ca:P ratio of the solid and also by a rise in the phosphorus content of the filtrate, as shown previously (*1*). A 2 p.p.m. treating solution does not furnish sufficient fluoride to exceed the solubility product of CaF_2. In dentin at 2,000 p.p.m., the F^- contributed by the formation of CaF_2 amounts to about 1.2 percent, and in enamel to about 1 percent.

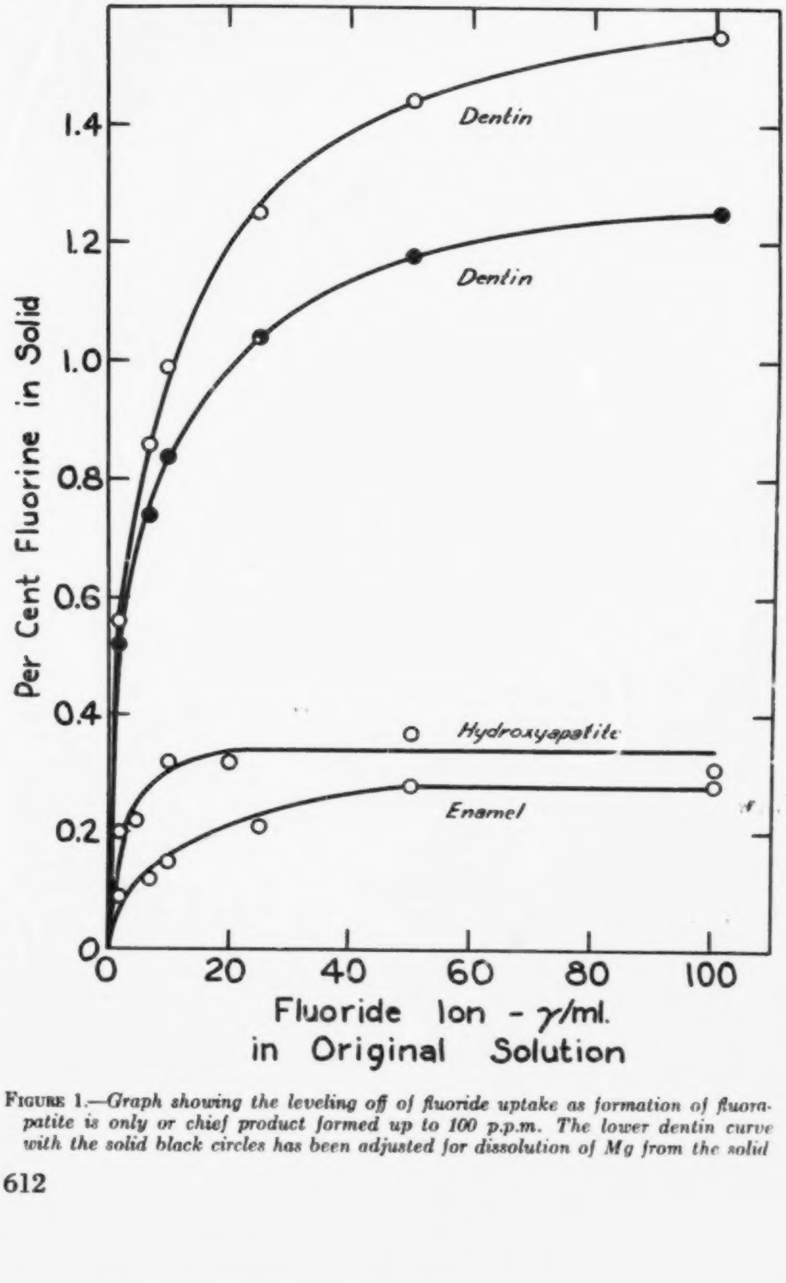

FIGURE 1.—*Graph showing the leveling off of fluoride uptake as formation of fluorapatite is only or chief product formed up to 100 p.p.m. The lower dentin curve with the solid black circles has been adjusted for dissolution of Mg from the solid*

(e) *Fluorapatite.*—The content of fluorapatite is determined by a fluoride balance. The summation of fluoride contributed by all the other mechanisms previously mentioned subtracted from the total fluoride content leaves a balance due to fluorapatite. This amounts to about 0.4 percent F^- at 2 p.p.m., and 1.2 percent F^- at 2,000 p.p.m. in the dentin, and reaches a maximum of about 0.2 percent in the enamel. In figure 1 are shown plots of the concentration of fluoride in the solid versus fluoride ion concentration in the original solutions taken from table II. The lower dentin curve has been adjusted by subtracting the MgF_2 content of the solid. The leveling off of these curves as they reach 0.2, 0.3, and 1.2 percent, respectively, is indicative of the formation of fluorapatite almost exclusively up to 100 p.p.m. Above this fluoride concentration, the curves again rise as CaF_2 is formed by double decomposition.

Reactions of enamel or of exposed dentin in vivo might differ qualitatively from in vitro reactions because of the presence of saliva and other substances. It would not be expected that magnesium would be dissolved from the tooth in most cases. However, the formation of fluorapatite and the direct adsorption of fluoride could take place, as could the double decomposition reaction to form calcium fluoride.

The mechanism of the inhibition of caries by fluorine is still a matter of conjecture. However, it seems reasonable to believe that chemical reactions in teeth exposed to fluorides, such as are found in natural and fluoridated drinking water, result in the formation of fluorapatite. Topically applied fluoride forms calcium fluoride (when a 2 percent NaF solution is employed), which is probably dissolved in time leaving fluorapatite and adsorbed fluoride. It is also possible that some of the adsorbed fluoride and some of the calcium fluoride is gradually converted to fluorapatite during the course of time.

SUMMARY

It would appear from the results of this study that at least five different reactions may take place between powdered dentin and enamel (or ions dissolved from them) and fluoride ion in vitro. The

approximate order of magnitude of these various reactions has been estimated. Additional evidence for the chemical nature of dentin and enamel has been presented. The reactions which may occur in teeth during the in vivo exposure to fluoride from fluoridated drinking water or by topical application are suggested.

We are indebted to Dr. R. C. Linkins of the National Institute of Dental Research for the preparation of the powdered samples of enamel and dentin and to Dr. Victor R. Dietz of the National Bureau of Standards, who obtained the sieve fractions and surface area measurements.

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PHOSPHATE EXCHANGE IN HYDROXYLAPATITE, ENAMEL, DENTIN, AND BONE. I. Mechanism of Exchange

By Harold G. McCann and Elizabeth H. Fath

In recent years a number of studies have been carried out on the exchange between mineralized tissues and synthetic hydroxylapatite and a solution containing radioactive phosphate or calcium. Neuman and Mulryan (*1*) have shown that there is a considerable difference between the exchange with ashed bone compared with that of fresh bone, the exchange with ashed bone coming to equilibrium very quickly, while that with fresh bone continues to exchange over a considerable period of time. This exchange with unashed bone has been explained by recrystallization. Dawson (*2*) has shown that Ca^{45} exchange with fresh adult ox bone comes to approximate equilibrium quickly, indicating that the reaction was one of surface exchange, with a gradual exchange thereafter, probably brought about by recrystallization. Recently Weikel, Neuman, and Feldman (*3*) have shown that there are actually three separate reactions which occur: exchange of ions in the bulk of the solution with ions in the hydration shell interchange of ions from the hydration shell with ions in the crystal surface, and an interchange of surface ions with subsurface ions.

In this paper we have been interested in the exchange of P^{32} with synthetic hydroxylapatite, enamel, dentin, and bone, and have indicated that the organic matter present in these tissues may play an important part in the rate of exchange in addition to the recrystallization process.

METHODS

Samples of synthetic hydroxylapatite, human enamel and dentin, and rat dentin and femur were obtained from studies reported previously (*4–6*).

In each of the experiments, 21 samples of each solid weighing 0.0050 gm were placed in 12 ml. centrifuge tubes. To each were added exactly 10 ml. of stock buffer solution. Buffer solution was 0.005 M, pH 7.0, KH_2PO_4-K_2HPO_4 containing P^{32} in an amount such that the experiment could be continued for 4 to 5 months (up to 380,000 c.p.m. per ml.). The samples were placed in a room maintained at constant temperature at 37° and were shaken at frequent intervals. The samples were removed in triplicate after periods of about 1, 7, 28, 56, 84, 112, and 140 days and centrifuged, and the solution was decanted and made to a convenient volume. The solid was washed once with water and centrifuged, and the wash water was added to the filtrate. The solid residue was dissolved in concentrated HCl and HNO_3, taken to dryness, redissolved in 0.5 ml. of 0.05 N HCl, and diluted to volume. Aliquots of all six samples from each time period were then counted by using standard procedures with a thin mica window Geiger tube and a scaling unit model No. 163 (Nuclear Instrument and Chemical Corporation).

The percent phosphorus exchanged was calculated from the counts in the solid: (counts in solid divided by original count in liquid) × (gm. of P in solution per gm. of P in solid) × 100 = percent exchange. The equation was obtained by the addition of the two equations: (counts in solid divided by original count in liquid) × gm. of P in solution = gm. of P exchanged. (Gm. of P exchanged × 100) divided by gm. of P in solid = percent P exchanged. The counts in the liquid samples were used to check the per cent recovery by adding to the counts in the solid and comparing the original count. It was found necessary to correct the counts taken after about 100 days for the presence of S^{35}, an impurity which increased to a significant amount because of the longer half life of 88 days.

RESULTS

The results with hydroxylapatite and human enamel are shown in figure 1. A

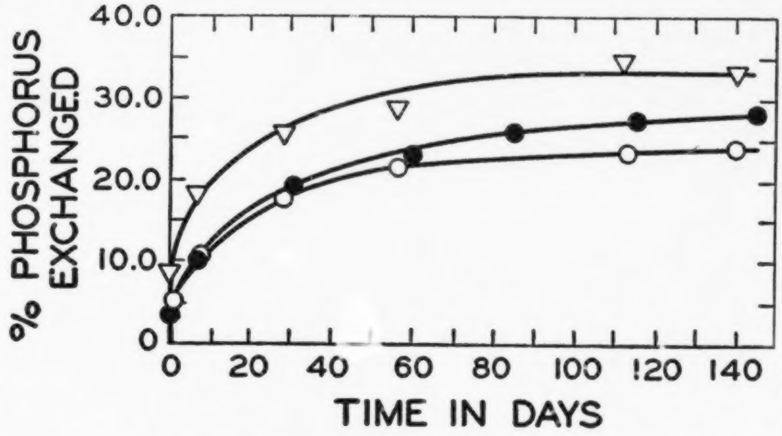

FIGURE 2.—*Transfer of P^{32}-labeled phosphate from solution to solid:* ○, *rat incisor dentin;* ●, *human dentin;* ▽, *rat femur.*

considerable part of the total exchange takes place within the first 24 hours, and then there is a gradual exchange which occurs during the next 4 months. In the case of hydroxylapatite the initial rapid exchange (24 hours) with P^{32} amounts to about 2.5 percent of the total phosphorus in a sample containing 17.63 percent phosphorus (*4*). If this 24 hour exchange is assumed to be limited to the surface, the average value of 0.238 mg. for surface P^{31} per square meter of surface area, as given by Olsen (7), may be used to calculate the surface area. This calculation results in a surface area of 18.6 sq.m. per gm. Previous study of this hydroxylapatite has shown that it reacts with fluoride ion to about 8.5 percent saturation of fluorine positions over the range 10 to 100 p.p.m. of F (*4*). Hendricks (*3*) has calculated that, if one assumes that the crystals are very flat on the prism faces and that this 8.5 percent fills all surface fluorine positions, then the crystals are 24 units of structure thick or about 200 A. This would correspond to a surface area of about 17 sq.m. per gm. The surface area of this particular sample as determined by low temperature nitrogen absorption (*8*) is 16.1 sq.m. per gm., so that the result would indicate that fluoride exchange with hydroxyl and the P^{32} exchange with P^{31} are restricted to the surface or a few layers near the surface. During an additional period of 4 months there is a slight additional amount of exchange, undoubtedly a thermal diffusion process which has been termed recrystallization (*1*).

The initial exchange with human enamel would indicate a surface area of about 5.5 sq.m. per gm., based on the average value for P^{31} as before. Since the low temperature nitrogen absorption value for this sample is about 1.5 sq.m. per gm., it is apparent that this initial exchange has penetrated several layers of the enamel or that in some way the phosphorus has reached layers not accessible to nitrogen at low temperatures. The latter seems the more likely since it is improbable that recrystallization would occur in 24 hours. considering the slow rate of recrystallization which takes place in the ensuing 4 months. If, for purposes of comparison of the reactions it is assumed that recrystallization has occurred to about the same extent during both the fluoride and P^{32} exchange, a calculated surface area from P^{32} exchange after 110 days compares closely with that obtained from the fluoride reaction after 4 months; that is, 16 sq.m. per gm. *versus* 15 sq.m. per gm. After the initial surface exchange, the rate is approximately the same in both enamel and hydroxylapatite samples, indicating a similar recrystallization rate. Probably the initial exchange through the enamel layers is affected by the small amount of organic material present.

In figure 2 are shown the exchange curves for bone and dentin. These curves show the typical gradual increase demonstrated by Neuman and Mulryan (*1*) for fresh unashed bone, although the slope of the curve as a whole is considerably different, partially because of the increased time over which the exchange was observed. These curves also have the same general slope as the curves for dentin and bone when fluoride exchange is plotted against time (*6*).

The surface of defatted bone has been shown to have a surface area of about 4 sq.m. per gm. by low temperature nitrogen absorption. This is similar to that of dentin (*5*). However, when this bone is steamed, the surface increases to about 67 sq.m. per gm., and, when glycol-ashed, the surface increases to over 100 sq.m. per gm. (*9*) The surface increase is attributed to the removal of organic substance from the pores of the inorganic skeletal material, since there is no detectable change in crystal size as shown by electron microscope studies (*10*). The surface area of the rat femur calculated from the P^{32} exchange is about 40 sq.m. per gm. after 24 hours. Apparently the solution phosphate or fluoride is able to penetrate the organic material and thus gain access to the inorganic crystals. Crystals of this size are only 2 to 4 unit cells thick (*11*), so that the inner portions should be able to recrystallize readily, and this is indicated by the extent of the reaction after 140 days. Glycol-ashed bone has been shown by Neuman and Mulryan (*1*) to exchange about 10 percent phosphorus in 48 hours and to exchange no further up to 20 days. Although the phosphorus content of this sample is not known exactly, it is probable that a surface area of about 70 sq.m. per gm. could be assumed from the P^{32} exchange, and this area might be as high as 100 sq.m. per gm. because of the lower surface phosphate content after the glycol-ashing treatment. After glycol ashing, bone would be sim-

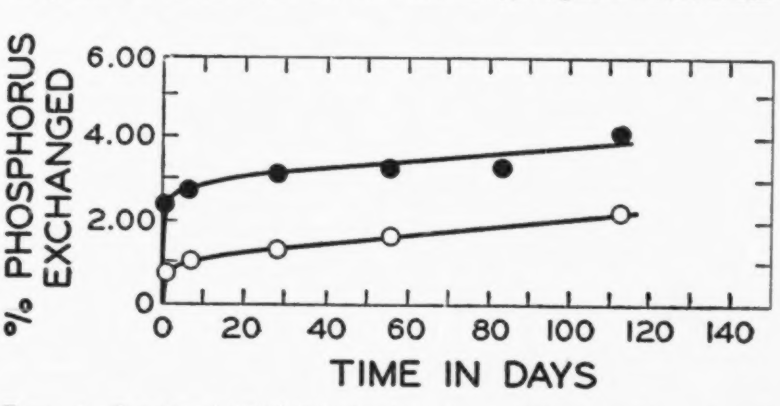

FIGURE 1.—*Transfer of P^{32}-labeled phosphate from solution to solid:* ○, *human enamel;* ●, *synthetic hydroxylapatite.*

ilar to hydroxylapatite and, hence, the major part of the exchange would be expected to take place within the 1st few hours. It is probable that, if the curve were continued for 2 to 4 months, there would be some further exchange attributable to recrystallization.

Dentin and bone behave similarly when the organic matrix is left intact and they are exposed to P^{32} or F exchange. They both have the same low temperature nitrogen absorption surface area with the organic matrix present (approximately 4 sq.m. per gm.), and the somewhat similar exchange of the dentin would indicate a surface area, if the organic matrix were removed, of about 50 sq.m. per gm. The gradual slope of the curves after 30 days is believed to be due entirely to recrystallization.

DISCUSSION

For some time one of the problems associated with the study of the mineralized tissues of the body has been the apparent irreversibility and relatively slow rate of isotope and fluoride fixation. This is in contrast to the rapid apparent equilibration of exchange with ashed tissues or synthetic hydroxylapatite. An initial rapid and reversible reaction can be accounted for by ionic exchange, but an additional mechanism is involved in the further slow and relatively irreversible reaction. This has been accounted for by recrystallization through thermal aging due to faults in the crystals (*1*).

Although the mechanism of recrystallization is undoubtedly one of the factors involved in the gradual and irreversible exchange process of mineralized tissues, it appears unwise to neglect one of the great differences between unashed bone and dentin and ashed bone or synthetic hydroxylapatite; that is, the large amount of organic matrix intimately bound up with the inorganic crystals in mineralized tissue. It seems likely that a large part of the relatively slow exchange in skeletal tissue can be explained by a diffusion of ions through the organic matrix so that the exchange can take place on the large surface area available on the crystals. Kolthoff and O'Brien (*12*), in experiments on the aging of AgBr, have shown that a coating of gelatin on the crystals inhibits but does not prevent the penetration of radioactive bromide with fresh AgBr. Once the ions reach the crystal surface, exchange would take place immediately. If this is true, the rate-determining step is the diffusion through the organic matrix. Once the surface of a crystal had exchanged completely, recrystallization could take place for further exchange within the crystals.

In a study of the exchange of P^{32} with ashed bone, dentin, and enamel over relatively short time periods, Hodge et al. (*13–15*) found that, when the amount of adsorbed P^{32} was plotted against the square root of the time, a linear relationship appeared. They concluded that P^{32} adsorption may involve some undefined diffusion process and that P^{32} exchanges with P^{31} on the crystal surfaces as speedily as diffusion permits. Their bone, however, was ashed, and hence the diffusion in this case was not through the organic matter.

The differences in the amount and rate of exchange with bones from different animals and of different ages could also be accounted for to a considerable extent by the variation in accessibility of surfaces due to variation in diffusibility through the organic matrix caused by variation in hydration, concentration of inorganic material, or other unknown structural differences.

Enamel contains little organic matter and behaves for the most part like hydroxylapatite with a relatively small surface area. It seems probable that the initial exchange, which is greater than can be accounted for by surface measurement by low temperature nitrogen absorption, might be due to the presence of this small but significant organic material which allows access to surfaces by solutions which are not available to nitrogen at low temperatures.

SUMMARY

The exchange of P^{32} with synthetic hydroxylapatite and with powdered and defatted enamel, dentin, and bone has been studied over a period of 110 to 140 days.

Although the surface area of fresh and ashed bone is approximately the same, P^{32} exchange with the ashed bone is practically complete in 48 hours, whereas with unashed bone a period of over a month is required before the exchange approaches completion. It is suggested that diffusion of ions through the organic matrix of dentin and bone is an important factor in the rate of exchange with fluoride ion and radioactive isotopes.

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PHOSPHATE EXCHANGE IN HYDROXYLAPATITE, ENAMEL, DENTIN, AND BONE. II. Effect of Fluoride on the Exchange

By Harold G. McCann and Elizabeth H. Fath

In previous studies of the reactions of fluoride ion with synthetic hydroxylapatite and powdered enamel and dentin *(1, 2)*, it was thought that low concentrations of fluoride ion might be affecting the rate of recrystallization of the apatites. Neuman et al. *(3)* found that fluoride impregnation had no effect on calcium and phosphate exchange of glycol-ashed bone.

The experiments described below were designed to study the effect of various amounts of fluoride on the phosphate exchange of various mineralized tissues and synthetic hydroxylapatite. The fluoride was introduced in the buffer solutions, and some of the tissues studied were previously fluoridated either in vitro or in vivo.

EXPERIMENTAL

Details of the phosphate exchange experiments with 0.005 M KH_2PO_4 were given in paper I of this series *(4)*. Samples of synthetic hydroxylapatite, human enamel and dentin, and rat dentin and femur, including samples fluoridated in vivo, were obtained from studies reported previously *(1, 2, 5)*. Tissues treated in vitro were exposed to an aqueous solution of NaF containing the indicated fluoride concentration for a period of 1 month at 37°.

RESULTS

Figure 1 shows the effect on P^{32} exchange of treating rat incisor dentin with 2, 10, and 100 p.p.m. of fluoride in the buffer as compared with use of buffer containing no fluoride. The curves obtained from 2 and 10 p.p.m. of F are higher than the control curve where no fluoride was used, indicating a more rapid rate of P^{32} exchange for the first 30 days and resulting in a larger overall amount of exchange. However, with 100 p.p.m. of fluoride less exchange was obtained than when no fluoride was used. Similar results were obtained when hydroxylapatite, enamel, human dentin, and rat femur were employed, although the overall amount of P^{32} exchange varied considerably, depending on the tissue. The final exchange after 4 months with 2 and 10 p.p.m. of F was always higher than with 100 p.p.m. of F, although their relative positions were sometimes reversed. With 100 p.p.m. of F the exchange was sometimes greater than when no fluoride was used, but the final exchange was always less than when either 2 or 10 p.p.m. of F were present in the buffer.

Figure 2 shows the effect of fluoride already present in the tissues added either in vivo or in vitro. The tissue treated in vivo contained 0.52 percent fluoride, the tissue treated in vitro, 0.87 percent fluoride. There is an initial increase in the rate of exchange and then a decreased amount of exchange as the reaction proceeds. The results were similar with human dentin treated in vitro with 2 and 100 p.p.m. of fluoride. Human enamel treated in vitro and rat femur treated both in vivo and in vitro exchanged less than the controls throughout the entire exchange period.

Figure 3 shows that fluoride in the buffer increases the rate and amount of exchange of tissues previously treated with fluoride in vitro. A similar effect was obtained with tissues previously treated with fluoride in vivo.

DISCUSSION

When fluoride is added to the buffer, it has a slight effect during the initial reaction which is restricted almost entirely

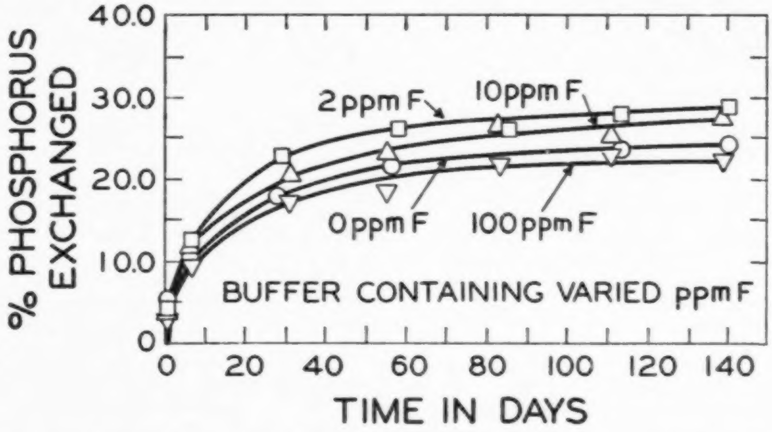

Figure 1.—*Effect on the exchange of P^{32}-labeled phosphate from buffer solution to solid rat incisor dentin when various concentrations of fluoride are added to the buffer.*

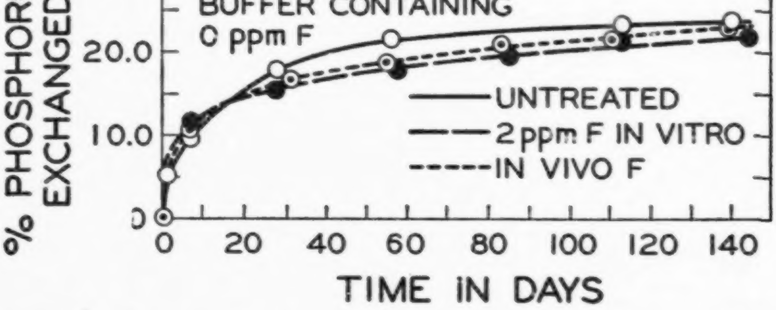

Figure 2.—*Effect of previous fluoride treatment of dentin on the exchange of P^{32}-labeled phosphate from buffer solution to solid rat incisor dentin.*

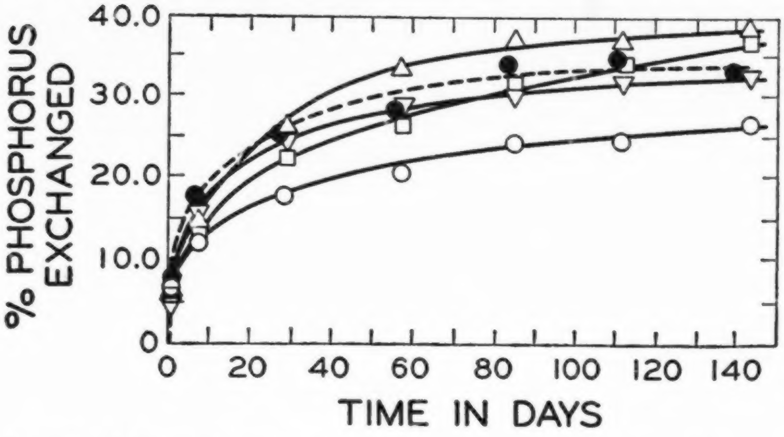

FIGURE 3.—*Effect on the exchange of P^{32}-labeled phosphate from buffer solution to previously fluoridated rat femora when various concentrations of fluoride are added to the buffer. The dashed line represents the original untreated femora with no fluoride in the buffer. Fluoride concentrations in the buffer are represented by:* ○, *0 p.p.m. of F;* □, *2 p.p.m. of F;* △, *10 p.p.m. of F;* ▽, *100 p.p.m. of F.*

to surface exchange. In most cases the effect is a slight increase in the amount of exchange. As the reaction proceeds, the exchange with hydroxylapatite and enamel must be through recrystallization. In the case of dentin and bone there is probably a slow surface exchange as the solution diffuses through the organic matrix *(4)*. This is combined with some recrystallization, and still later the exchange is due entirely to this latter effect. In all cases as recrystallization begins to take place, the fluoride increases the rate and amount of exchange to a considerable extent. It seems probable that a fluorapatite crystallization center, in contrast to the hydroxylapatite, would induce a greater stability to the crystal, which would tend to increase the recrystallization of the particles because of the tendency to reach the most stable thermodynamic state.

The increase in the rate of exchange of P^{32} caused by increasing fluoride concentrations falls off markedly at 100 p.p.m. of F. At higher sodium fluoride concentrations the sodium ion may substitute for calcium ions *(6)*, possibly accounting for the decrease at a concentration of 100 p.p.m. of F. Thus sometimes the 2 p.p.m. of F and sometimes the 10 p.p.m. of F curve are uppermost, showing the greatest amount of exchange, but the 100 p.p.m. of F curve is always lower and sometimes even drops below the 0 p.p.m. of F control curve.

With fluoride already present in the tissues, the exchange rate sometimes increased slightly at first, but the rate of subsequent exchange was less than that of the control, probably because of decreased solubility. Recrystallization because of the fluoride has been completed before addition of the P^{32} and the effect of the fluoride ions is to decrease the solubility, slowing, rather than increasing, the P^{32} exchange rate. This increased exchange during the first 24 hours may be caused by a release of fluoride ions into the solution. This would correspond to the increased exchange of P^{32} with previously fluoridated tissues when additional fluoride is added to the buffer.

In a previous paper *(5)* the similarity in behavior of dentin and femur treated in vivo and in vitro with fluoride in respect to solubility and hydroxyl exchange was given as evidence for the formation of fluorapatite in vivo. The similarity in P^{32} exchange of the tissues fluoridated in vivo and in vitro is additional evidence for the formation of fluorapatite in mineralized tissue when fluoride ion is ingested.

SUMMARY

The simulatenous use of fluoride ion and P^{32} has shown that fluoride ion increases the rate of exchange of P^{32} with synthetic hydroxylapatite, human enamel and dentin, and rat dentin and bone. The rate is also increased by the presence of fluoride ion in the buffer when enamel, dentin, and bone have been previously treated with fluoride either in vivo or in vitro. The rate of exchange of P^{32} with previously fluoridated enamel, dentin, or bone, compared with the corresponding untreated control, is decreased.

Additional evidence for the formation of fluorapatite in mineralized tissues has been presented.

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Water Defluoridation

The removal of excessive quantities of fluoride from a community drinking water is a necessary procedure for the prevention of endemic mottled enamel. Defluoridation of a natural fluoride water should extend only to a level of 1.0 p.p.m. of fluoride, optimum for the effective control of dental caries.

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REMOVAL OF FLUORIDE FROM WATER

By Elias Elvove

In considering the problem of the removal of fluoride from drinking water, it appeared desirable to find a substance the use of which would not leave in the water any element or group not ordinarily present in appreciable quantity; and which, likewise, would not impair the potability of the water by increasing any ordinary constituent to an undesirable concentration. Although the field from which to choose was thus narrowed, three substances were found that appeared promising. Other substances have been reported in the literature; but since the present paper is intended as a brief note, it is not deemed necessary to discuss here the related literature on the subject. Some references to other published methods for the removal of fluoride from drinking water are, however, included in the list of references at the end of this paper. These are tricalcium phosphate, magnesium oxide, and magnesium hydroxide. Scott, Kimberly, Van Horn, Ey, and Waring have reported (Am. Water Works Asso., January 1937, pp. 9-25) the reduction of the fluoride content of magnesium-containing waters by applying the ordinary lime softening treatment but over-treating with the lime to a causticity of about 2 grains per gallon.

A report describing methods for the removal of fluoride from water by means of tricalcium phosphate, magnesium oxide, or magnesium hydroxide, was forwarded by the writer to the Surgeon General on June 26, 1936, for consideration of the advisability of applying for letters patent, to protect the public interest and the right of any citizen of the United States to use these methods without payment of royalties.

The results obtained with tricalcium phosphate and with magnesium hydroxide were qualitatively similar to those obtained with magnesium oxide. Since, however, the question of cost is very important, and since, at present, magnesium oxide appears as the least expensive, experiments have been conducted mostly with this substance. Calcined magnesite is quoted at about 3 cents per pound ($60 per ton) and tricalcium phosphate at 6.5 cents per pound in the March 1937 Market Report of Industrial and Engineering Chemistry. No comparable price on magnesium hydroxide is quoted. Light magnesium oxide complying with purity requirements of the United States Pharmacopedia is quoted at 36 cents per pound.

It seems reasonable to expect that different grades of commercial magnesium oxide would show different degrees of fluoride-removing power. Preliminary experiments indicated that this is actually the case; and while it appears probable that a magnesium oxide could be prepared for this special purpose which would have a fluoride-removing power considerably above that of the ordinary commercial grades, it is questionable whether from the point of view of cost, it might not be more economical to utilize a commercially inexpensive magnesium oxide even though its fluoride-removing power is, per given weight, relatively lower.

The results reported in this paper have been obtained with two different grades of magnesium oxide. The grade referred to as calcined magnesite was obtained from a business concern which uses it in the construction of cement floors. The other grade was a commercial sample of light magnesium oxide similar to that of the United States Pharmacopœia. In both cases, the magnesium oxides were used in the finely-divided condition as they are ordinarily sold.

One mode of operation may be illustrated by the following described experiments. The fluoride-containing water to be treated was introduced into tall bottles so as nearly to fill them to the beginning of the curved portion. The quantity of the magnesium oxide used corresponded to one ounce per gallon of water in each bottle. The magnesium oxide and water were then actively agitated, by means of a current of air, for about half an hour. After complete settling, about three-fourths of the column of water was siphoned off, replaced by a fresh supply of the fluoride-containing water, and the process repeated.

The water used in these experiments was prepared by adding sufficient sodium fluoride to distilled water to make its fluoride content 5 parts per million. On the basis of quantitative epidemiological studies *(1, 2)*, such a fluoride concentration in a drinking water would be expected to produce a high incidence of mottled enamel when used continuously by children during the susceptible age period. The fluoride was estimated with the aid of the zirconium-alizarin reagent *(3)*. The results obtained are summarized in the accompanying tables.

Since the cost of treatment would probably vary with different localities, with the varying cost of the magnesium oxide, with the varying composition of the dissolved solids in the water, the cost of possible methods of reactivation, or the reduction of the original cost by utilizing the magnesium oxide for other

TABLE 1.—*Comparative results with a commercial calcined magnesite and a commercial light magnesium oxide*

Number of times the MgO was used in this series (A)	Fluoride (F) content before treatment (B)	Fluoride after treatment with the calcined magnesite (C)	Fluoride removed [1] (D)	Fluoride after treatment with the light magnesium oxide (E)	Fluoride removed (F)
	Parts per million				
1	5	0.2	4.8	0.2	4.8
2	5	.7	4.3	.4	4.6
3	5	.7	4.3	.6	4.4
4	5	1.7	3.3	.9	4.1
5	5	1.9	3.1	1.3	3.7
6	5	2.5	2.5	1.3	3.7
7	5	2.1	2.9	1.7	3.3
8	5	2.7	2.3	1.8	3.2
9	5	2.2	2.8	1.9	3.1
10	5	2.1	2.9	1.7	3.3
11	5	2.1	2.9	1.7	3.3
12	5	3.5	1.5	1.9	3.1
13	5	3.0	2.0	2.0	3.0
14	5	4.5	.5	2.3	2.7
15	5	4.0	1.0	2.2	2.8
16	5	4.2	.8	3.0	2.0
17	5	4.0	1.0	3.0	2.0

[1] Application of a similar procedure to a natural water showing about 419 p. p. m. of total solids, 58 SiO_2, 47 Ca, 45 Mg, 356 HCO_3, 48 SO_4, 2.8 NO_3, 10 Cl, and 3.5 p. p. m. of fluoride, reduced the fluoride content to 0.6, 0.6, and 0.9 p. p. m. in the first, second, and third runs, respectively.

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[2] This usually required standing overnight.

TABLE 2.—*Results with a composite water after a second treatment, using a commercial calcined magnesite*

Number of times the MgO was used in this series (A)	Fluoride (F) content before any treatment (B)	Fluoride of the composite[1] sample after one treatment with 4-times-used calcined magnesite (table 1) (C)	Residual fluoride after a second treatment (with a fresh portion of the magnesite) (D)	Fluoride removed by first treatment (B−C) (E)	Fluoride removed by second treatment (C−D) (F)	Total fluoride removed by the two treatments (E+F) (G)
	Parts per million					
1	5	2.9	0.7	2.1	2.2	4.3
2			.6		2.3	4.4
3			1.1		1.8	3.9
4			1.0		1.9	4.0
5			.8		2.1	4.2
6			.8		2.1	4.2
7			.9		2.0	4.1
8			1.1		1.8	3.9
9			1.0		1.9	4.0
10			1.2		1.7	3.8
11			1.5		1.4	3.5
Average			1.0		1.9	4.0

[1] This composite represented a mixture of the water from runs 5 to 17 (table 1, column C).

TABLE 3.—*Results with a composite water after a second treatment, using a commercial light magnesium oxide*

Number of times the MgO was used in this series (A)	Fluoride (F) content before any treatment (B)	Fluoride of the composite[1] sample after one treatment with 5-times-used light magnesium oxide (table 1) (C)	Residual fluoride after a second treatment (with a fresh portion of the oxide) (D)	Fluoride removed by first treatment (B−C) (E)	Fluoride removed by second treatment (C−D) (F)	Total fluoride removed by the two treatments (E+F) (G)
	Parts per million					
1	5	2.1	0.6	2.9	1.5	4.4
2			.7		1.4	4.3
3			.7		1.4	4.3
4			.6		1.5	4.4
5			.7		1.4	4.3
6			.7		1.4	4.3
7			.6		1.5	4.4
8			.7		1.4	4.3
9			.6		1.5	4.4
10			.6		1.5	4.4
11			.9		1.3	4.2
Average			.7		1.4	4.3

[1] This composite represented a mixture of the water from runs 6 to 17 (table 1, column E).

purposes after its use for fluoride removal, we can consider this question at present only partially and in general terms. The treatment with magnesium oxide leaves a slight caustic alkalinity which would have to be neutralized (with carbon dioxide). This, therefore, is another item of expense to be considered. We may, however, consider particularly the important factor of the efficiency of a given specimen of the magnesium oxide as a fluoride-remover. If we take the gallon and pound as the units for our calculations, we may summarize the above results as follows:

Table 1 shows that, in the case of the calcined magnesite, the fluoride removal in the first three runs was 4.3 to 4.8 parts per million, yielding water with residual fluoride of less than 1 p.p.m. (0.2 to 0.7); but if the waters were mixed, that from the fourth run could be added and the composite sample would still show less than 1 p.p.m. (0.8). In the case of the light magnesium oxide, the first four runs yielded water with residual fluoride of less than 1 p.p.m. (0.2 to 0.9); but if the waters were mixed, those from the fifth, sixth, and seventh runs could be added and the composite sample would still show less than 1 p.p.m. (0.9). The fifth to the seventeenth runs, in the case of the calcined magnesite, yielded a composite sample that showed a fluoride removal of about 2.1 p.p.m.; while in the case of the light magnesium oxide, the sixth to the seventeenth runs yielded a composite water that showed a fluoride removal of about 2.9 p.p.m. Using a composite water which had received one treatment, the residual fluoride in the mixture obtained from 11 runs with a second portion of magnesium oxide (tables 2 and 3) was reduced to about 1 p.p.m. or less (about 1 in the case of the calcined magnesite and 0.7 in the case of the light magnesium oxide).

The fluoride-removing power of the magnesium oxides, however, was not yet completely exhausted. In the subsequent 25 runs with the magnesium oxide that had already been used in 17 runs (table 1), there was a further removal of fluoride, corresponding to an average of about 0.8 p.p.m. in the case of the calcined magnesite and to about 1.8 p.p.m. in the case of the light magnesium oxide; and when about two-fifths of each of these waters were then treated a second time with magnesium oxide that had already been used in 11 runs (tables 2 and 3), there was a further average reduction of about 1.5 p.p.m., or a total fluoride removal of about 2.3 p.p.m. in the case of the calcined magnesite (table 4). In the case of the light magnesium oxide, the corresponding figures were about 1.9 and 3.7 p.p.m., respectively (table 5).

If we do not consider the indicated residual fluoride-removing power of the used magnesium oxides (tables 4 and 5) and limit our calculations to the quantities of water in which the fluoride was reduced from 5 to about 1 p.p.m. in not exceeding two treatments (tables 1, 2, and 3), the above results show that, when the operation was carried out on a basis of 1 ounce of the magnesium oxide per gallon of water in the treatment container, the two treatments (using a total of 2 ounces per gallon) were effective in reducing the fluoride to not exceeding about 1 p.p.m. for a quantity of water corresponding to about 11 gallons when using the calcined magnesite and to about 12 gallons when using the light magnesium oxide. These figures were derived as follows: In the case of the calcined magnesite, the mixed water from the first 4 runs (table 1, column C) did not require a second treatment; but since only three-fourth of the column of water was siphoned off after each run, only the water of the first run may be

Table 4.—*Showing the residual fluoride-removing power of the used calcined magnesites*

Number of times the 11-times-used calcined magnesite (table 2) was used in this series (A)	Fluoride (F) content before any treatment (B)	Fluoride content of the mixed water obtained from 25 subsequent runs with the 17-times-used calcined magnesite (table 1) (C)	Residual fluoride in (C) after treatment with the 11-times-used calcined magnesite (table 2) (D)	Average fluoride first removed by the 17-times-used calcined magnesite in 25 subsequent runs (B−C) (E)	Fluoride subsequently removed by the 11-times-used (table 2) calcined magnesite (C−D) (F)	Total fluoride removed in two treatments (by the 17-times-used and 11-times-used) calcined magnesites (E+F) (G)
	Parts per million					
1	5	4.2	2.3	0.8	1.9	2.7
2			2.2		2.0	2.8
3			2.3		1.9	2.7
4			2.2		2.0	2.8
5			2.7		1.5	2.3
6			3.1		1.1	1.9
7			2.8		1.4	2.2
8			3.1		1.1	1.9
9			3.2		1.0	1.8
10			3.0		1.2	2.0
Average			2.7		1.5	2.3

Table 5.—*Showing the residual fluoride-removing power of the used light magnesium oxides*

Number of times the 11-times-used light magnesium oxide (table 3) was used in this series (A)	Fluoride (F) content before any treatment (B)	Fluoride content of the mixed water obtained from 25 subsequent runs with the 17-times-used light magnesium oxide (table 1) (C)	Residual fluoride in (C) after treatment with the 11-times-used light magnesium oxide (table 3) (D)	Average fluoride first removed by the 17-times-used light magnesium oxide in 25 subsequent runs (B−C) (E)	Fluoride subsequently removed by the 11-times-used (table 3) light magnesium oxide (C−D) (F)	Total fluoride removed in two treatments (by the 17-times-used and 11-times-used) light magnesium oxides (E+F) (G)
	Parts per million					
1	5	3.2	1.1	1.8	2.1	3.9
2			1.1		2.1	3.9
3			1.0		2.2	4.0
4			1.0		2.2	4.0
5			1.1		2.1	3.9
6			1.4		1.8	3.6
7			1.7		1.5	3.3
8			2.0		1.2	3.0
9			1.5		1.7	3.5
10						
11			1.0		2.2	4.0
Average			1.2		1.9	3.7

considered as representing a whole gallon of treated water, while the water obtained from the 3 subsequent runs corresponded to only 2.25 gallons (0.75 × 3) of treated water. Similarly, the water from the first run of table 2 corresponded to a whole gallon, but the contribution of the subsequent 10 runs corresponded to only 7.5 gallons (0.75 × 10). The total quantity of water with a residual fluoride of not exceeding about 1 p.p.m., therefore, corresponded to 11.75 gallons (1 + 2.25 + 1 + 7.5), or about 11 gallons in round numbers.

Similarly in the case of the light magnesium oxide, the contribution of sufficiently treated water from the first 5 runs of table 1 represented 4 gallons (1 + 3); and the water from the second treatment (table 3) represented 8.5 gallons (1 + 7.5). The total in this case, therefore, corresponded to 12.5 gallons (4 + 8.5), or about 12 gallons in round numbers. On this basis, 1 pound of the calcined magnesite would serve to treat about 88 gallons and 1 pound of the light magnesium oxide would be sufficient to treat about 96 gallons of such water.

It is to be noted, however, that the above treatment has been applied to a fluoride concentration considerably higher than is ordinarily found in endemic areas *(4)*. As indicated in tables 2 and 3, the cost of treatment would be lower when the fluoride concentration is less than 5 p.p.m. (a larger number of runs with the same magnesium oxide yielding water with a residual fluoride of not exceeding about 1 p.p.m. when starting with 2.1 or 2.9 instead of 5 p.p.m.).

As has been stated, the magnesium oxides used were utilized in the condition as sold for their ordinary uses. This eliminates an increase of cost on account of preparatory treatment. Besides using a chemical which is comparatively inexpensive to start with, it is believed that, in the case of the calcined magnesite particularly, since it is used extensively in building operations and for various other purposes, there is opportunity for realizing a salvage value for the material after its use as a fluoride-remover for drinking water, and thus indirectly reducing the cost of treatment. The comparatively small amount of fluoride or other constituents of the water that will have been absorbed by or removed with the magnesium oxide probably would not interfere with its subsequent utilization. In this connection, it may be interesting to note that Nagai and Takahara *(5)* have recently proposed the addition of small amounts of fluoride to portland cement raw mixtures for promoting combination. Likewise, Shaw and Shaw *(6)* have recommended as beneficial the presence of a small proportion of fluoride as magnesium silico-fluoride in the preparation of concretes, mortars, and plaster from medium or lightly burned dolomite.

SUMMARY

Results obtained indicate that fluoride can be removed from water with the aid of tricalcium phosphate, magnesium oxide, or magnesium hydroxide. Since the question of cost is very important, and since, at present, magnesium oxide appears to be the least expensive, experiments have been conducted mostly with this substance. Different grades of commercial magnesium oxide showed different degrees of fluoride-removing power. Although a commercial light magnesium oxide was found more efficient as a fluoride-remover, per given weight, than a commercial calcined magnesite, its greater efficiency was not proportional to its present higher cost. From the point of view of economical operation, much work remains to be done. It is pointed out, however, that in the case of the calcined magnesite particularly since it is used extensively in building operations and for various other purposes, it may be possible to utilize the material after its availability for fluoride removal has been exhausted and thus indirectly reduce the cost of treatment. Such utilization would add another advantage to the advantages of its commercial availability in large quantities and comparatively low initial cost.

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METHODS OF REMOVING FLUORIDES FROM WATER

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INTRODUCTION

Fluorides in drinking water can be beneficial or detrimental depending on their concentration. Recent epidemiological studies have indicated that there is an inverse relationship between fluorides and dental caries experience, (*19, 22, 24*). The promise which this relationship holds for mass control of dental decay, however, hinges on the fact that an optimal caries prophylactic effect of the fluoride is obtained within the range of 1.0 to 1.5 parts per million (p.p.m.). Greater concentrations are associated with a hypoplasia of the teeth known as mottled enamel or dental fluorosis. It is the purpose of this presentation to examine current methods for removing excessive fluorides from communal water supplies in order to prevent the occurrence of dental fluorosis. Inasmuch as present evidence suggests that the removal process should not effect a fluoride reduction below 1.0 p.p.m. in order that the beneficial effect of caries inhibition may be retained, this discussion will be concerned with the defluorination of water to the optimal limit.

Near the beginning of the 20th century mottled enamel had been noted and described (*25*). Despite the intensive researches of several investigators, however, it was not until 1931 that its cause was correctly determined and announced, almost simultaneously, by Churchill (*14*), Smith (*49*), and Velu (*59*). Since the discovery that fluorides were the cause of mottled enamel in the permanent teeth of children, the problem of removing exces fluorides from drinking water has been studied widely.

In 1935, Dean and Elvove (*16–18, 21*) began to publish the results of their epidemiological studies relating to minimal concentrations of fluorides sufficient to produce mottling. They found that a fluoride concentration of 1.0 p.p.m. or less in a communal water supply produced no significant mottling of the teeth of children who had been using this water continuously. With higher concentrations, mottling was progressively more severe and above 6.0 p.p.m. almost all children were afflicted. In addition to mottling at this high concentration, gross calcification defects and attrition of the enamel were observed. For this reason, the dental defects caused by fluorides in drinking water are better termed dental fluorosis.

After the cause of dental fluorosis was discovered, several methods were devised for removing excessive fluorides from water. Nevertheless, at the present time (excepting some lime softening plants) only one community is known to be utilizing equipment specifically installed to reduce the fluoride content of its water supply. The lack of application of preventive measures maintains despite the undesirable disfigurement associated with fluorosis, the increased costs of dental care and the fact that the number of persons using water containing excessive fluorides (over 1.5 p.p.m.) exceeds one million persons in over 500 communities (*58*). This situation may be attributed to a combination of several factors, most important of which are the high capital and predicted operating costs of the removal processes and the complexity of the operating procedures.

It is impossible or impracticable in many communities to change the source of supply to one having a satisfactory fluoride concentration. Dental studies have been made in three communities, however, which previously used water high in fluorides and then changed to other sources having little or no fluorides (*23*). The change resulted in no additional cases of fluorosis (*20, 40*). No community has as yet removed excessive fluorides from its water and subsequently measured the results in terms of a reduction in the incidence of fluorosis.

Fluoride Removal Methods

The methods utilizing the fluoride exchange properties of the apatites, such as those involving the use of the constituents of bone, the ion exchange principle, and those depending on the sorptive properties of aluminum compounds, appear to show the most promise for removing excess fluorides from water. In addition, where the fluoride concentration is not very high (less than 4.0 p.p.m.), processes involving the concurrent removal of magnesium are indicated, if hardness reduction is also contemplated.

Calcium Phosphates

The use of bone for removing fluorides is based on the long known affinity of bone for fluorides. Probably the most plausible theory of the chemistry of this affinity may be explained on the basis of the anion exchange properties of apatites. The carbonate radical in the apatite comprising bone $nCa_3(PO_4) \cdot CaCO_3$ is replaced by the fluorides in the water, forming an insoluble fluorapatite *(50)*. In the regeneration of the material with sodium hydroxide the fluorapatite probably becomes an hydroxy-apatite and the fluorides are removed in the form of soluble sodium fluoride. The hydroxy-apatite subsequently becomes available as an exchange material by the replacement of its hydroxy radical with fluoride.

One method of preparing bone for use as a fluoride removing agent *(51)* involves boiling to remove the fat and most of the protein. It is then crushed and boiled in a solution of sodium hydroxide. The caustic is removed by thorough washing and finally is neutralized with hydrochloric acid. The material is again washed and then dried and graded for fineness. Home filters in which the prepared bone is utilized are available but there have been some objections to the taste of the treated water. Based on the expense of renewing the bone media in home filters, the cost of using this method in a municipal plant would be exorbitant (approximately $25,000 per m.g.). All chemical cost estimates, except where otherwise noted, are based on a fluoride ion (F^-) reduction from 6.0 p.p.m. to 1.0 p.p.m. at current (1947) chemical quotations. It is reported *(50)* that the material can be regenerated successfully by a sodium hydroxide and acid wash method, similar to that used in its preparation. Data are not available on the removal capacity of the bone after it has been subjected to numerous regenerative cycles when used with waters naturally high in fluorides and other mineral contaminants.

A similar process utilizes virgin bone black (animal charcoal) which is essentially tricalcium phosphate and carbon *(13)*. In its limited use in household filter units, the material is discarded when its exchange capacity is exhausted. In a municipal installation, the cost of this procedure would be excessive ($5,000 per m.g.). Regeneration of the material has been effected with solutions of tri-sodium phosphate and mono-sodium phosphate but the exchange capacity of the bed is reduced by 12 percent after the first regeneration.

The principal chemical constituent of bone, tricalcium phosphate, can be prepared from phosphoric acid and milk of lime. The product consists essentially of tricalcium phosphate and its hydroxy-apatite. Because it forms a gelatinous floc in water, fluoride removal is probably accomplished by absorption together with the formation of a fluorapatite *(1, 26, 28)*.

A porous, granular type of tricalcium phosphate has been developed for use in contact filters *(3)*. When the fluoride removal capacity of the bed is exhausted, the material can be regenerated with a 1 per cent sodium hydroxide solution. About one pound of caustic soda is required for each cu. ft. of tricalcium phosphate *(30)*. The causticity of the bed is then neutralized by thorough washing with water and with a carbon dioxide solution *(7)*. The use of carbon dioxide for this purpose, rather than hydrochloric acid as originally proposed, greatly increases the life of the bed and considerably reduces attrition losses *(8)*. The rated removal capacity of tricalcium phosphate is 300 grains of fluoride per cu. ft. but this capacity decreases about 3 percent for each 100 p.p.m. sulfates present in the water. The iron content of the water must be reduced to 0.10 p.p.m. in order to prevent clogging of the filter. A water treatment plant using this material has been operated since 1937 *(60)*. The fluoride content of the raw water varied from 2.0 to 14.0 p.p.m. and averaged about 8.3 p.p.m. The effluent contained an average of 0.6 p.p.m. The chemical costs for the fluoride removal process at this plant totaled about $130 per m.g. in 1944.

Tricalcium phosphate can also be formed from ortho-phosphoric acid and lime within the mixing chamber of the conventional type of treatment plant (mixing flocculation, settling and filtration) *(37, 38)*. The tricalcium phosphate thus formed must be removed continuously from the water and discarded. The chemical cost of this process amounts to approximately $245 per m.g. A similar material for fluoride removal, trimagnesium phosphate, has been suggested *(2)*, but is reported to be less efficient and more expensive than tricalcium phosphate.

Adsorption with Aluminum Compounds

In addition to the constituents of bone for fluoride removals, a large number of other materials have been tested. These materials include aluminum sulfate, sodium aluminate, zeolites, bauxite, silica gel, sodium silicate, ferric fluoride, lime *(11)*, and various adsorbent clays and earths *(41)*. With the exception of aluminum sulfate, all of these materials have been found to be impracticable probably because of the very low initial concentration of the fluoride ion, and in some instances because an undesirable proportion of the reagent remains in the water due to the excessive amounts of chemicals required. Aluminum sulfate and other aluminum salts have been used in combination with insoluble compounds in contact beds or as constituents of floc which is subsequently removed by settling and filtration. The fluorides might be removed by the formation of an aluminum fluoride complex or by adsorption on the floc. In addition to the fluoride removal characteristics of the aluminum compounds, they are relatively inexpensive, easy to use, and can be combined advantageously with aids to coagulation such as clays and activated silica. The results reported on the effectiveness of aluminum hydroxide floc formed from aluminum sulphate and a source of alkalinity vary considerably. The variations can be attributed to such factors as the following: different raw waters with varying induced fluoride concentrations were used; the speed of adding the reagent and methods of mixing were different *(12)*; the times of contact varied; the pH was not the same; and, not all of the effluents were filtered. In general, however, 4.0 p.p.m. fluorides are removed from water when hydrous aluminum sulfate is used in concentrations of 335 p.p.m. *(11)* 430 p.p.m. *(34)* or 513 p.p.m. *(47)*. According to one investigator, it required 891 p.p.m. aluminum sulfate to reduce fluorides from 6.0 to 1.0 p.p.m. *(47)*. While according to another *(34, 35)* 860 p.p.m. were required to obtain a reduction from 8.5 to 1.0 p.p.m. In order to obtain maximum fluoride reductions, optimum hydrogen ion concentration is necessary but the pH can be controlled in treatment plants by adjusting the chemical feeders.

Fluoride removal experiments in which aluminum sulfate and coagulation aids (clays and activated silica) were used together with alkalin solutions for pH control, were conducted recently in the Industrial Hygiene Laboratories at the National Institute of Health and in pilot plants at Great Falls, Md. (Potomac River raw water), and at Cincinnati, Ohio (Cincinnati City water) *(39)*. Fluoride concentrations (*10*) were determined by using the Scott modification (*48*) of the Sanchis procedure (*46*) and the Lamar adaptation (*36*) with a spectrophotometer. The results with the conventional type of plant at Great Falls (mixing, flucculation, settling, and filtration) indicated that fluorides can be reduced in two steps (6.0 to 3.5 p.p.m. and from 3.5 to 1.0 p.p.m.) using in each step 100 p.p.m. aluminum sulfate, 100 p.p.m. clay, and about 25 p.p.m. lime. In an improvised experimental sludge-blanket type plant (precipitator) in Cincinnati approximately 25 percent more fluorides were removed using the same chemical dosages as at Great Falls. When activated silica (Baylis Sol) was used in conjunction with alum, clay, and soda ash, a further reduction in fluorides was noted (*4, 5, 6, 32, 33*). Based on the quantities of chemicals used in the conventional pilot plant the cost is estimated to be about $35 per m.g. The process is not as efficient with highly mineralized ground water in which excessive fluorides are naturally present. The reason for decreased efficiency under the latter conditions is not known.

Another method utilizing aluminum salts involves contact beds of insoluble material impregnated with aluminum compounds. The earliest report (*43*) suggested soaking filter beds of sand or diatomaceous earth with the precipitated hydroxide of aluminum sulfate and an alkali. The data available on the fluoride removal efficiencies of this method are not sufficient to serve as a basis for estimating costs.

A similar method involves pickling base exchange materials (*54*) (natural or synthetic zeolites) or naturally adsorptive materials (*55*) in aluminum salt solutions. Although these pickling solutions are highly concentrated, five hours of contact are required before the exchange material has been converted to an anion exchange compound. The aluminum ion is presumed to have entered into the chemical structure of the material and reversed its exchange mechanism. When the bed is exhausted, it can be regenerated with soluble aluminum salts. This method is the least efficient of those in which aluminum soaking is involved (*44*).

Another method involving aluminum salts and utilizing contact beds is based on the formation of insoluble compounds in the presence of excess aluminum ions. The insoluble compounds thus formed are alleged to possess a fluoride removal capacity several times that of materials of the base exchange group. The superior performance is attributed to the much higher concentration of aluminum salts in solution and to the concomitant increase in adsorbed uncombined aluminum ions (*56*). The insoluble compounds can be made from either sodium silicate, barium chloride, sodium phosphate, ferrous sulfate or titanium chloride in the presence of excess aluminum salts. The precipitates formed are washed, dried and graded. Regeneration is accomplished by washing the bed with solutions of either aluminum salts or dilute mineral acids or alkalies. No data are available on the fluoride-removing capacity of this method or of its efficiency after repeated regenerations. Until other commercial uses can be found for the insoluble materials used in this method, their cost for fluoride removal will probably be too high for use in municipal installations.

Dehydrated aluminum oxide (calcined alumina) (*15*) or partially hydrated alumina (*31*) have been suggested for use in contact beds. The calcined alumina (activated alumina) has been tried in field tests (*29, 52*) and rated as fairly good with respect to fluoride removal efficiency. When the bed becomes exhausted the fluorides are removed by washing with a dilute caustic solution and neutralized with a dilute acid solution. Chemicals for regenerating the materials used in this method amount to $275 per m.g. Because tricalcium phosphates have a better removal capacity and a lower regeneration cost than activated alumina, the latter is no longer advocated for municipal treatment plant use.

Other hydrated metallic oxides have also been suggested for removing fluorides. These include ferric oxide, chromium borate, mixed oxides of iron and manganese, bauxite and bog iron ore. All can be regenerated to varying degrees but ferric oxide is probably the most efficient (*31*). Using caustic soda and carbon dioxide solutions for regeneration, chemicals cost approximately $250 per m.g.

Ion Exchange Processes

While the compounds which are subjected to aluminum solution soaking probably involve a combination of ion exchange and adsorption principles in fluoride removal, several additional products are claimed to involve predominately ion exchange processes. One of these is made from barium or ferric chloride and silicic acid with the formation of a complex metal chloride silicate. It is claimed (*53*) that the fluorides are removed from water when in contact with this material by exchange with the chloride ions. Regeneration of the materials has not been advocated and no data are available on capacities or costs. Another compound is obtained from asphaltic materials heat treated with an alkali (*57*). Fluorides are presumed to be removed by anion exchange with the hydroxide. Regeneration is accomplished with an alkali but capacities and costs have not been determined.

Recently an organic resinous anion exchange material made from various diaminobenzenes or phenols and formaldehyde has been announced (*45*). It is alleged that this material is somewhat selective for fluorides, removing in addition only iron, copper or other metals yielding insoluble hydroxides (*44*). The material is first conditioned with a 4 percent soda ash solution, neutralized with a dilute acid wash (500 p.p.m. hydrochloric acid) and then soaked with a 4 percent solution of aluminum sulfate. The exchange capacity is based on 1,240 grains of fluorides per cu. ft. Chemical costs for regeneration amount to about $90 per m.g. Further tests on this material to determine the effect of higher concentrations of radicals commonly found in ground waters and the possibility of utilizing other regenerating chemicals are now in progress.

Other Methods

The use of lime for fluoride reduction was known soon after the cause of fluorosis was discovered (*11*). In a study of the results of lime softening plants in Ohio (*47*), it was determined that fluoride reduction with lime was actually a function of the amount of magnesium removed. When waters were fluorinated with sodium fluoride and treated with magnesias, it was demonstrated that the magnesia first becomes partially hydrated, then magnesium fluoride and sodium hydroxide are formed by metathesis and finally the magnesium fluoride is attached to the magnesia, forming perhaps an oxyfluoride (*61*). The results of samples examined from the softening plants indicated that the quantity of fluorides removed approximately equaled the product of 7 percent of the initial fluoride and the square root of the amount of magnesium removed. Between 45 and 65 p.p.m. magnesium must be removed to reduce the fluoride one p.p.m. Theoretically, if a residual fluoride of 1.0 p.p.m. is desired, 100 p.p.m. magnesium must be removed if the initial fluoride content is 3.3 p.p.m. A magnesium concentration of this magnitude is rare in most high fluoride waters. Consequently employment of this method would involve the addition of magnesium preferably in the form of dolomitic lime, calcined magnesite or activated magnesia (*9, 26, 27*). For average waters containing 6.0 p.p.m. fluoride and relatively high magnesium (20 p.p.m.), about eight tons of chemicals would be required per m.g. (carbon dioxide for pre- and post-carbonation, dolomitic lime and aluminum sulfate). The chemical costs would aggregate $120 per m.g. Despite this relatively high cost and the large quantities of chemicals required (involving principally sludge disposal problems), this method appears to be particularly applicable in the treatment of hard waters which require softening and which contain less than 4.0 p.p.m. fluorides. A refinement of this process provides a source of carbon dioxide for pre-carbonation (*9*), which is necessary to convert the magnesia to magnesium carbonate. The carbon dioxide is produced from conversion of bicarbonates by passing the water through hydrogen exchange materials.

Other chemicals and processes have been reported, many of which proved to be ineffective or impracticable. Activated carbon is quite efficient and its use is based on sound chemical principles (*42*). Almost complete fluoride removal is possible, however, only when the pH of the raw water is less than 3.0. No fluoride is removed at a pH of 8.0 or higher. Regeneration is possible with weak alkaline and acid solutions although no reports have been made as to the removal capacity of the carbon after repeated regenerations.

About one-half the fluorides originally present in water are removed using dosages of approximately 2,000 p.p.m. of either Wyoming bentonites Fuller's earths, celite, or silica gel. To obtain this removal, however, the pH of the raw water is reduced to 2.5. In no case is any fluoride removed when the pH of the water is 8.3 (*41*). Because of the inefficiency of these materials and their pH requirements their use is impracticable for fluoride removals.

SUMMARY AND CONCLUSIONS

More than a million persons in over 500 communities in the United States are now using public water supplies containing in excess of 1.5 p.p.m. fluorides. The disfiguring dental condition which is caused by the use of such waters for drinking purposes can be prevented in future populations of these communities by removing the excess fluorides from the communal water supplies.

Most of the removal processes now available appear to be either too expensive to operate or too complicated for routine application by the average small water treatment plant operator. An urgent need is the development of a process comparable to the lime-soda ash softening process in operating costs and simplicity.

The problem of choosing the most practical method of defluorination for a particular supply is difficult, because of the almost complete absence of operational data. The choice of method for the accomplishment of a similar purpose as in other treatment processes, depends, by and large, on the rate of water consumption and on the characteristics of the raw water. For large municipal plants which treat waters requiring a reduction in hardness, it is advisable, in general, to reduce the fluorides as much as possible by the lime softening process. In some cases it would be economical to balance the costs of pre-carbonation and sludge disposal against the addition of a limited quantity of magnesium compounds. Excess fluorides remaining after this process could be removed in gravity contact filters or by an aluminum-clay floc, with subsequent separation of the floc from the water.

In small plants where softening is not desired, pressure contact filters appear to be more economical. Where the water is hard, lime softening is indicated up to a raw water fluoride content of about 4.0 p.p.m. If the fluoride exceeds this figure in hard waters, the remaining fluorides can be removed by contact filters.

The tricalcium phosphates and the resinous ion exchangers, when used in contact filters, appear to have the highest exchange capacities for fluorides. Furthermore, the chemical cost for regeneration of these materials is relatively economical.

It is evident from this examination of fluoride removal methods that our current knowledge of the chemical processes involved is very limited and that additional fundamental research in this field is needed. Further, the practical usefulness of the available methods and those now undeveloped must be tested on a pilot plant scale, and their relative worth under varying conditions must be determined. Until this information is available, endemic fluorosis will continue to be a dental hazard to a large population in many communities in this country.

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DEFLUORIDATION OF MUNICIPAL WATER SUPPLIES

By F. J. Maier

Although an optimum amount of fluoride in water is a valuable means for promoting dental health, excessive concentrations are associated with a hypoplasia of the teeth known as dental fluorosis, the severity depending on the concentration. For example, children who have continuously used water containing 6.0 p.p.m. or more fluoride are, without exception, afflicted with mottled enamel of the permanent teeth. In addition, many of them have gross calcification defects and loss of enamel through attrition. Such defects are permanent and frequently cannot be ameliorated.

Despite the undesirable disfigurement associated with fluorosis, the increased cost of dental care, and the fact that the number of persons using water containing excessive fluoride (over 1.5 p.p.m.) exceeds a total of one million in more than 500 communities, only five plants designed specifically for fluoride removal have been built. This failure to utilize an available preventive measure probably is due to the reported high capital and chemical costs and the complexity of the operating procedures in various defluoridation processes.

Ever since the cause of fluorosis and the methods for preventing it became known, a practicable means for reducing excessive amounts of fluoride in water has been sought. To be within the reach of smaller communities, the defluoridation process, it was believed, should make use of equipment comparable to that in conventional treatment plants, should have operating costs within the range of those for softening plants, and should be simple enough to be carried on by local employees with little training.

In selecting a site for demonstrating such processes in a full-scale plant, the

U.S. Public Health Service looked for a community having: [1] a water supply containing at least 6.0 p.p.m. fluoride; [2] no realistic prospect of obtaining another, low-fluoride source of water; [3] a population of approximately 2,000 persons, which would yield a minimum significant number in each age group for fluorosis measurements; [4] practically universal metering; [5] a willingness to contribute at least half the capital cost of the plant. The USPHS, because of the fact that the data obtained from such a plant would be of value to other communities throughout the country, would agree to pay the operating cost for the first 10 years.

The Texas Health Department suggested that Bartlett, Tex., an agricultural center in Bell and Williamson counties with a population of 1,760, would meet these requirements. Its water is obtained from two wells, 1,000 feet deep, each delivering approximately 220 g.p.m. A typical analysis is shown in table 1.

TABLE 1.—*Composition of Bartlett water*

Item	Amount ppm
Total solids	2,000
Total alkalinity	400
Phenolphthalein alkalinity	0
Total hardness (as $CaCO_3$)	176
Calcium	41
Magnesium	18
Iron	0.3
Manganese	0.05 –
Sodium	560
Silica (SiO_2)	25
Color	0
Turbidity	0
Carbonate	0
Bicarbonate	488
Sulfate	500
Chloride	350
Fluoride	8.0
Nitrate	0.04 –
Odor	H_2S
pH	7.8

The selection of Bartlett was considered particularly fortunate for demonstration purposes because the town had a water with the highest fluoride content of any public supply in the country, as well as a very high mineral content. If this supply could be treated successfully, the chances were that many others could be. In addition, the city council and public utilities staff were most cooperative and competent.

In order to determine the best process available, an extensive study was made (*1*) and numerous tests were conducted in the USPHS laboratory. In evaluating the published methods for removing fluoride from water, some were found to be worthless, others were impossibly complicated, and most, although they could be used with throw-away media on faucet filters, were too expensive for municipal treatment plants. For the Bartlett plant, the processes available were narrowed down to the use of calcium phosphates, anion-exchange resins, magnesias, or aluminum compounds—materials that can be employed as exchange media and regenerated by chemical treatment.

CALCIUM PHOSPHATES

It has long been known that the bone has an affinity for fluoride. Perhaps the most logical explanation for this reaction involves the anion-exchange properties of apatites. The carbonate radical of the apatite comprising bone, $Ca_9(PO_4)_6 \cdot CaCO_3$, is replaced by fluoride, forming the insoluble fluorapatite (*2*). The caustic soda used in regeneration converts the fluorapatite to hydroxy-apatite, with the fluoride removed as soluble sodium fluoride. The hydroxy form then becomes the exchange material, with the hydroxy radical replaced by fluoride.

The use of bone was one of the earliest methods suggested for defluoridating water. The Smiths of Arizona (who share, with Churchill, the discovery that fluoride in water is the cause of fluorosis) suggested the use of degreased, caustic-and-acid-treated bone. This material was not considered for Bartlett because of its high cost—an expenditure of $25,000 would have been required—and because of reports of objections to the taste of water treated by faucet defluoridizers.

A similar material, virgin bone black (animal charcoal), is essentially tricalcium phosphate and carbon (*3*). First reports suggested the use of trisodium phosphate and monosodium phosphate as a regenerant. Because this process reduced the capacity of the bed by 12 percent after the first regeneration, however, it was not considered for Bartlett. With the development of a caustic soda-carbonic acid regeneration, bone char was selected to replace the original synthetic apatite at another plant under study, at Britton, S.D. This material has been in use for less than a year. Its capacity appears to be approximately 450 grains of fluoride (as F) per cubic foot, while chemical costs are estimated at $50 per million gallons of water treated. Chemical cost estimates in this paper are based on 1953 prices for the quantities most likely to be used.

The principal chemical ingredient of bone, tricalcium phosphate, can be obtained from the reaction between phosphoric acid and lime. If the reaction is carefully controlled, the product is essentially tricalcium phosphate and hydroxyapatite. It can be formed in solution within the mixing basins of conventional treatment (flocculation, settling, and filtration) plants (*4*). Based on pilot plant studies (*5*), the chemical cost of this process at Bartlett would have amounted to $230 per million gallons of water treated. A similar material, trimagnesium phosphate, has been tried but would cost more than tricalcium phosphate because of its lower fluoride removal capacity.

Tricalcium phosphate (synthetic hydroxyapatite) can also be obtained in a porous, relatively insoluble form by controlling the concentrations of the phosphoric acid and lime solutions. It can be fed into water as a powder, followed by settling and filtration (*6*), with the spent material discarded. A more economical method utilizes a larger particle size (20-40 mesh) in a contact filter (7). Various improvements in regeneration and extensive laboratory studies resulted in the use of this medium in the first full-scale defluoridation plants, at Climax, Colo., in 1937 (*8*), and at Scooba, Miss., in 1940. Because of prior experience with this material, the USPHS adopted the process at its Britton, S.D., plant in 1948. The water there contains 6.7 p.p.m. fluorides, which was reduced to an equivalent of 1.5 p.p.m. Both of the earlier plants, at Climax and Scooba, have now been abandoned, while, at Britton, because of excessive attrition losses (42 percent per year), this medium was replaced with bone char during February 1953.

ION EXCHANGERS

The removal of fluorides with the various calcium phosphates and aluminum and magnesium compounds involves a combination of ion exchange and sorption. An additional group of defluoridation products depends on ion-exchange properties entirely. A large number of these materials have been developd, many of them patented, and their relative merits have been evaluated (*1*).

A resinous anion exchanger, Amberlite IR-4B, a product of Rohm and Haas Co., Philadelphia, was at first believed to be the most promising of this group. Later studies showed that its capacity was derived entirely from the precipitated aluminum oxide formed in the column during alum regeneration. This floc so thoroughly restricted the columnar flow as to make the process impracticable. The same company developed a strongly basic anion exchanger, XE-75, for fluoride removal, intended for use with salt regeneration. A recently published report (*9*) indicates that this material has

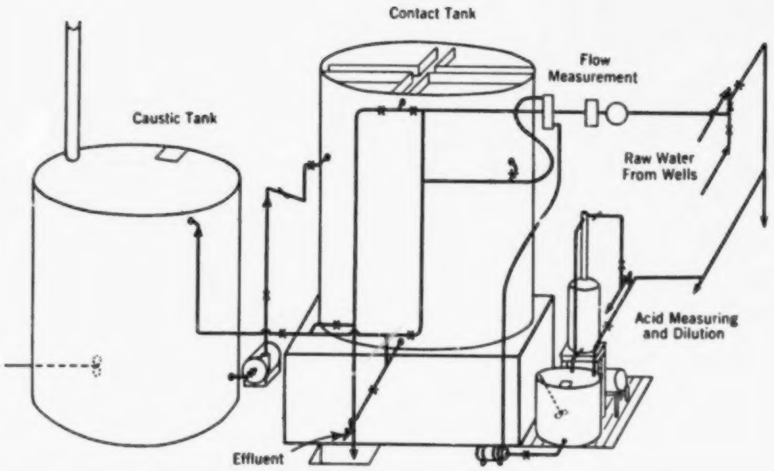

FIGURE 1.—*Bartlett defluoridation plant. The steel contact tank is 11 feet in diameter and 11½-feet high. The caustic tank holds 6,000 gallons. The installation has a designed flow-through rate of 400 g.p.m.*

a fluoride removal capacity of approximately 40 grains per cubic foot of resin when the raw water, as at Bartlett, has a fluoride—total anion ratio of 0.0147. The cost of salt alone, based on countercurrent regeneration of 1.0 pounds of sodium chloride per cubic foot of resin, would amount to $200 per million gallons of water treated at Bartlett.

The capacity of this exchanger increases rapidly with higher fluoride—total anion ratios, but most high-fluoride waters have a very low ratio. At Britton, for instance, this ratio is 0.0083, which would reduce the capacity to approximately 20 grains per cubic foot.

Proposals have been made to deionize high fluoride waters almost completely and blend the effluent with raw water to produce a final fluoride of approximately 1.0 p.p.m. Using some of the best exchange materials available (*10*), the cost of chemicals for this process at Bartlett would be approximately $850 per million gallons.

MAGNESIA

The use of lime was recognized as a means for reducing fluoride in water soon after the cause of fluorosis was discovered (*11*). Later Scott (*12*), in his study of results at lime-softening plants in Ohio, showed that the decrease in fluoride was a function of the magnesium removed. The amount of fluoride removed approximately equaled 7 percent of the initial fluoride times the square root of the quantity of magnesium removed. Between 45 and 65 p.p.m. magnesium must be removed to realize a 1-p.p.m. reduction in fluoride. Based on this formula, if 1 p.p.m. fluoride is desired in the treated water, 100 p.p.m. magnesium must be removed when the initial fluoride content is 3.3 p.p.m. Magnesium concentrations of this magnitude are rare in most high-fluoride waters. When the natural magnesium content is too low for adequate fluoride reduction, dolomitic lime, magnesia (*6*), or magnesium sulfate may be dissolved, converted to magnesium hydroxide, and settled. It has been demonstrated (*13*) that the magnesia becomes partially hydrated, then magnesium fluoride and sodium hydroxide are formed by metathesis, and finally the magnesium fluoride is recombined with the magnesia, perhaps forming an oxyfluoride.

Because of the large quantities of chemicals required, this process is adaptable primarily to low-fluoride (3-4 p.p.m. or less) waters requiring softening. Such a plant has been built in Bloomdale, Ohio, where fluorides are reduced from 2.2 to 1.2 p.p.m. using only the existing magnesium content of the water (*14*). At Bartlett, more than 10 tons of chemicals (carbon dioxide for pre- and post-carbonation, alum, and dolomitic lime), costing approximately $300, would have been required per million gallons of water. In addition, the necessary plant facilities, comprising dissolvers, reaction tanks, and filters, would have cost more than the contact filter type of plant actually adopted. Moreover, the problem of chemical and sludge handling was complicated by the fact that the plant was situated in the center of town.

ALUMINUM COMPOUNDS

Many other materials were tested, including aluminum sulfate, sodium aluminate, zeolites, bauxite, silica gel, sodium silicate, ferric fluoride, and lime (*11*), as well as various absorbent clays and earths (*15*). Except for aluminum sulfate, all of these have been found impracticable for full-scale plants.

Filter alum was recognized as having a relatively high fluoride absorption capacity and was desirable from the standpoint of cost and availability (*16-18*). The capacity can be increased with coagulant aids such as activated silica and clays, but, unfortunately, other anions in the water reduce it. At Bartlett, such interference would have required 900 p.p.m. alum to remove 7 p.p.m. fluoride, at a cost of $190 per million gallons of water for the alum, clay, and silica. Furthermore, this dose of alum would have raised the sulfate content to an even more undesirable level.

Dehydrated aluminum oxide, calcined alumina, and partially hydrated alumina were suggested for use in contact beds. The calcined (activated) alumina had been tried in field tests, in the USPHS laboratory, and at its pilot plant at Bartlett. With Bartlett water, the 10 cu. ft. pilot plant indicated that the fluoride removal capaciy of alumina was 514 grains (as F) per cubic foot. The alumina selected was 28-48 mesh, with a density-in-place of 50 pounds per cubic foot, and is commercially available as a desiccant.

Based on pilot plant data, a full-scale plant, containing 500 cu. ft. of alumina, was designed for Bartlett, assuming a 400-g.p.m. flow-through rate and one regeneration per week during the winter. The plant, housed in a 37×24-foot building provided by the city, consists of a steel contact tank, 11 feet in diameter and 11½ feet high, complete with underdrains, wash troughs, and a plastic ("Saran," a product of Dow Chemical Co., Midland, Mich.) caustic distributor; a 6,000-gallon caustic solution tank with mixer and pump; and acid dilution tanks with metering and proportioning equipment (fig. 1).

The two 220-g.p.m. well pumps, discharging directly into the contact tank, are controlled automatically by a float switch on the two interconnected, treated-water, ground storage tanks. A float valve on the contact tank actuates a mercury switch connected to the well pumps to prevent overflowing. The float switch also actuates the diaphragm valve on the effluent line, which controls the rate of flow and prevents the bed from draining when the pumps stop.

After regeneration, either well pump is started and is thereafter automatically controlled by the level in the storage tanks. When an equivalent of 1.0 p.p.m. fluoride in the treated water is reached, the cycle is stopped and the

TABLE 2.—*Calculation of equivalent fluoride*

1	2	3	4	5	6	7
Quantity Treated Between Fluoride Readings *1,000 gal*	Cumulative Quantity Treated *1,000 gal*	Fluoride Reading *ppm*	Avg of 2 Readings *ppm*	Col. 1 × Col. 4	Summation of Col. 5	Equivalent Fluoride, Col. 6 / Col. 2 *ppm*
0	0	4.4				
10	10	2.9	3.65	36.5	36.5	3.7
5	15	2.5	2.70	13.5	50.0	3.3
14	29	1.5	2.00	28.0	78.0	2.7
21	50	0.8	1.15	24.2	102.2	2.0
25	75	0.5	0.65	16.3	118.5	1.6
25	100	0.3	0.40	10.0	128.5	1.3
50	150	0.25	0.28	14.0	142.5	0.95
87	237	0.3	0.28	24.4	166.9	0.7
63	300	0.8	0.55	34.7	201.6	0.7
50	350	2.0	1.40	70.0	271.6	0.8
50	400	3.6	2.80	140.0	411.6	1.0

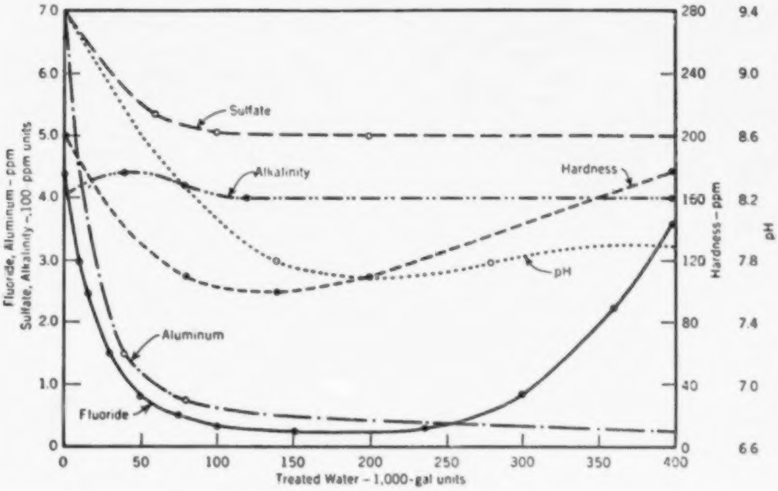

FIGURE 2.—*Concentration of various ions during typical cycle (fluoride, aluminum, and sulfate ion concentrations are shown, as well as alkalinity, hardness and pH).*

bed regenerated. The method used to calculate the point at which the 1.0-p.p.m. fluoride equivalent is attained is illustrated in table 2. The variations of some of the other ions in the treated water throughout a typical cycle are shown in figure 2.

REGENERATION OF ALUMINA

The purposes of regeneration are to clean the bed (by backwashing), to remove the accumulated fluorides with a caustic solution, to collect as strong a spent caustic solution as possible for the next regeneration, and to remove the residual caustic from the bed with water and dilute acid rinses.

Before regeneration is started, the caustic solution is made up to approximately 1 percent strength. The concentration of caustic in the returned solution from the previous cycle is determined by titration. The 6,000 gallons of return caustic solution contains 125-140 pounds of caustic. The difference between this figure and 500 pounds is added to the caustic tank.

The acid-handling equipment (fig. 3) was designed so that no vessel is subjected to pressure and no manual operation involving the acid is required. The sulfuric acid is diluted in two steps—to 15 percent and then to 0.05*N*. The first step is accomplished by using an aspirator-induced vacuum to transfer 284 pounds (18.55 gallons) of acid from the shipping drum to the measuring tank. The acid then flows into and is mixed with 194 gallons of water in a 252-gallon lead-lined tank. During regeneration, this solution is mixed with 61 times its volume of water to produce a 0.05*N* solution.

The bed is first backwashed, primarily to remove accumulated sand, precipitated iron, and oil sludge (from the oil-lubricated well pump bearings). A 30 percent bed expansion requires 5.7 g.p.m. per square foot, or a maximum of 542 g.p.m. Approximately 8,000 gallons of treated water is used during the 15-minute backwashing. Raw water is employed for the remainder of the regeneration.

Caustic solution is then pumped through the bed at a rate of 235 g.p.m. Even though the void volume of 500 cu. ft. of medium represents 1,140 gallons, approximately 5,500 gallons of caustic solution must be passed through the bed before a caustic alkalinity appears in the waste effluent. This is probably due to a chromatographic phenomenon involving progressive adsorption of hydroxyl ions. The volume of the first caustic rinse is chosen so that the largest amount of accumulated fluorides is discarded while maintaining the accompanying caustic strength at a minimum. As shown in figure 4, when 2,500 gallons of rinse water has been used, most of the fluorides have been discarded, while the caustic strength is just beginning to rise. At this point, the caustic return is started and continued until the caustic tank is filled.

A second caustic rinse is used to reduce the alkalinity of the bed to a point at which the further use of raw water alone is uneconomical. The volume (1,500–2,500 gallons) is determined by noting where the alkalinity curve begins to become asymptotic. After this point has been reached, acid is used to complete the neutralization of the bed.

The 15 percent acid previously prepared is diluted with the raw water and distributed over the bed. Generally 12,900 gallons of water at 220 g.p.m. is required to produce a 0.05*N* acid solution.

A final rinse of 20,000 gallons is used to lower the initial fluoride reading and reduce the aluminum concentration. The total water required for regeneration amounts to 55,000 gallons, of which only 8,000 gallons is treated water.

CONCLUSION

Regular operation of the Bartlett plant started March 11, 1952. The cost of chemicals to remove 7.0 p.p.m. fluorides with a 400,000-gallon cycle is $52 per million gallons. Equipment costs were $11,360, including installation. The alumina bed cost $4,000 in place. One man, working part time, operates

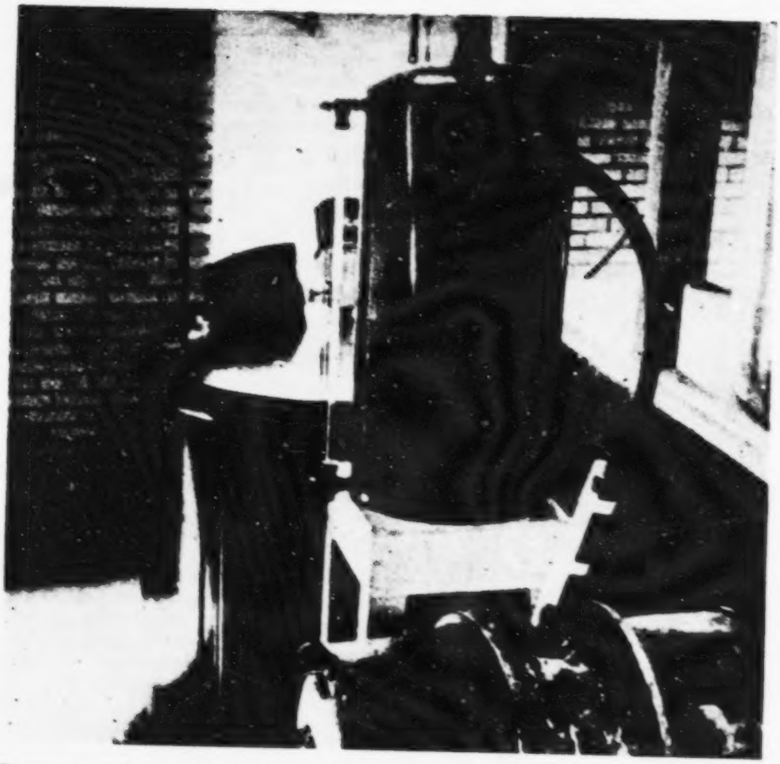

Figure 3.—*Sulfuric acid dilution equipment (an aspirator-induced vacuum is used to transfer the acid from the shipping drum (foreground) to the measuring tank (center). The acid is then diluted to 15 percent in the 252-gallon lead-lined tank at the rear).*

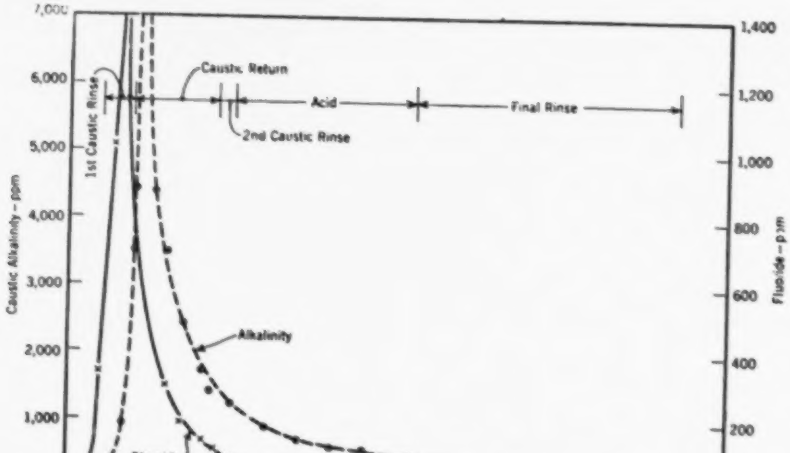

Figure 4.—*Progress of caustic alkalinity and fluoride during regeneration (in the return caustic, the average caustic alkalinity is 3,450 p.p.m., while the average fluoride is 300 p.p.m.).*

the plant and performs the necessary chemical tests, including fluoride distillations and photometer readings. Changes are being made continually in order to improve the efficiency of regeneration and the fluoride removal capacity of the aluminia.

The experience being obtained with the various types of defluoridation plants now in operation should provide a basis for adopting this preventive public health measure in the many places where fluorosis is now a problem. Plants have been designed so that local employees can run them successfully. The capital and operating costs are believed to be within the resources of many communities using high-fluoride water.

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PARTIAL DEFLUORIDATION OF WATER

By F. J. Maier

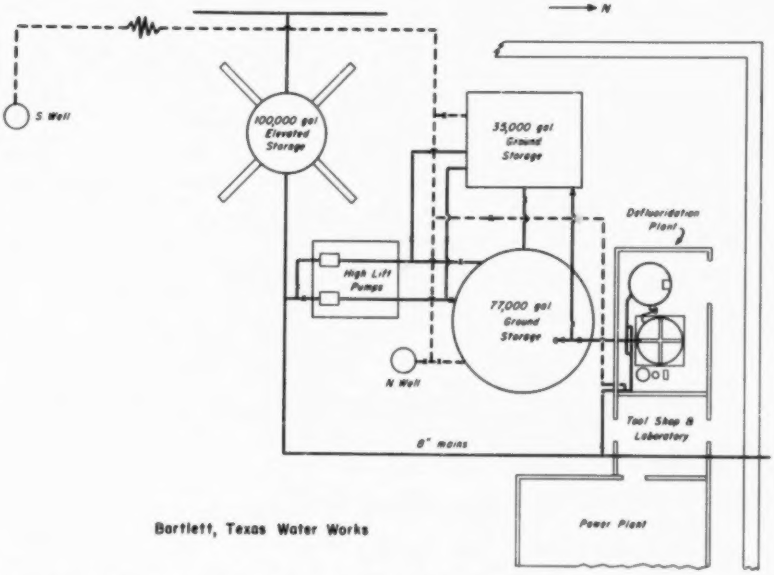
S Well
100,000 gal Elevated Storage
35,000 gal Ground Storage
→ N
Defluoridation Plant
High Lift Pumps
77,000 gal Ground Storage
N Well
8" mains
Tool Shop & Laboratory
Power Plant
Bartlett, Texas Water Works

FIGURE 1.—*Layout of Bartlett, Tex., water plant, with activated alumina contact unit for reducing fluorides to a safe level. Solid lines show treated water.*

For more than 10 years public water supplies containing excessive fluorides have been treated in order to reduce the fluoride level to the optimum (0.6 to 1.5 mg/L fluoride depending on the local temperature). Early in 1960 the teeth of the children living in communities served by such treated supplies were reexamined. It was found that the widespread fluorosis had been stopped among children brought up on defluoridated water and no single instance of severe fluorosis was observed.

The undesirable disfigurement and increased cost of dental care associated with fluorosis was known for several decades prior to 1931 when the cause was discovered. Soon afterward, it was shown that the severity of fluorosis was directly related to the fluoride concentration in the water used during the period of permanent tooth calcification. Children continuously exposed to water containing about 5 mg/L or more fluorides are invariably afflicted with mottled enamel of the permanent teeth; many of these children have gross calcification defects which weaken the enamel and cause eventual loss of the teeth through attrition.

Even though methods for the removal of excessive fluorides from water were published soon after the cause of fluorosis was discovered, little, if any, progress was made in the reduction of the incidence of fluorosis through these methods. This lack of acceptance of available measures for preventing fluorosis is probably due to the alleged excessive costs of treatment plants, the costs of operating such plants, and the complexity of the operating procedures. Actually the experience we have so far gained in the design, construction and operation of various processes for defluoridating water indicates that these allegations are no longer tenable.

At the present time there are approximately 4.1 million people living in 1,200 communities which are served by water sources containing fluorides in excess of the optimum range. In 264 of these communities (in which 690,000 people live) the fluoride levels are at least three times the optimum a (level at which about 10 percent of children exhibit fluorosis which has been classified as moderate or severe). Despite the widespread use of such waters as sources of public supplies only three plants designed specifically for fluoride removal are now operating. Two of these were designed and built, and are now being operated, by the U.S. Public Health Service while the third is operated by the U.S. Army.

DEFLUORIDATION METHODS

At present three defluoridation methods have proven practicable under varying conditions of raw water quality and availability of treatment chemicals. Two of these methods involve the use of either activated alumina or bone char in the form of an insoluble granular media which removes the fluorides as the water percolates through them. The media are periodically regenerated by chemical treatment when they become saturated with the fluoride that was removed from the water. In the third method magnesium may be added to the water in the form of dolomitic lime. It is removed, after absorbing the fluorides, by passing the water through settling basins and filters and is then discarded.

Activated alumina is used at the plant at Bartlett, Texas, and at the Army plant at Camp Irwin, near Barstow, California. It is also used in several experimental home defluoridation units. This media is available from several aluminum manufacturers in various mesh sizes of granules and degrees of purity. Activated alumina is commonly used as a desiccant, particularly in air conditioning equipment. For our purpose, it is used in mesh sizes between 28 and 48. It is currently selling for 15 cents per pound (FOB manufacturer) and weighs, in place, 50 pounds per cubic foot. The Bartlett plant contains 500 cubic feet of media in a standard, circular, steel, filter tank 11 feet in diameter and 11½ feet high. It is fitted with a steel pipe-grid underdrain, steel wash water troughs and a 4-inch plastic acid and caustic distributor. There is a 12-inch layer of graded gravel underlying 60 inches of media. The regeneration equipment includes a 6,000 gallon caustic solution tank with electric mixer and pump and a system for measuring, diluting and metering acid solutions. The plant is capable of treating 400 gpm of Bartlett well water (Edwards limestone formation) and was designed to reduce the fluoride from 8.0 mg/L to an average of 1.0 mg/L. The variations in some common ions which were analyzed in the treated water from a typical cycle are shown in Figure 2.

Either or both of the two 220-gpm well pumps discharge into the contact tank. They are controlled automatically by a float switch in the two interconnected, treated-water, ground-storage tanks. Overflowing of the contact tank is prevented by a float-valve-actuated mercury switch wired into the well pump starters. The float valve also positions the diaphragm-actuated rate-of-flow controller.

When between 400,000 and 500,000 gallons of 8.0 mg/L fluoride water have been treated the media becomes partially saturated with fluorides and must be regenerated. Regeneration of the activated alumina is intended primarily to remove these accumulated fluorides and to prepare the contact bed for the subsequent cycle. Regeneration consists of various steps designed to remove, by backwashing, the accumulated solids that had been strained from the water; to remove (by means of a weak caustic solution) the fluorides that had been adsorbed by the media; and to neutralize the residual caustic with weak acid and water rinses.

Before regeneration is started, a caustic solution of approximately 1 percent strength is prepared by dissolving 400 pounds of flake sodium hydroxide in 5,000 gallons of water. Equipment is available for returning the spent caustic for resuse but this has been found to be impracticable because of excessive contamination by other ions. Plans have been completed for changing to liquid (50 percent) caustic which is considerably more economical. The liquid caustic will be stored in the caustic solution tank and fed into the rinse water line with a gear pump.

The acid is applied to the media as a 0.05 N solution. This is obtained by diluting the 66° Baumé sulfuric acid in two steps—to 15 percent and then to 0.05 normal. An aspirator-induced vacuum is used to remove 284 pounds of acid from the shipping drum to a measuring tank. The acid flows into and is mixed with 194 gallons of water in a 252 gallon lead-lined tank. During regeneration this mixture is diluted with 61 times its volume of water. Lately, the 15 percent acid has been fed with a diaphragm-type chemical solution feeder into the raw water to reduce the pH to 7.2 (from a raw water pH of 7.8). This has not only increased cycle lengths and improved the removal capacity of the media but has drastically reduced the amount of acid required during regeneration.

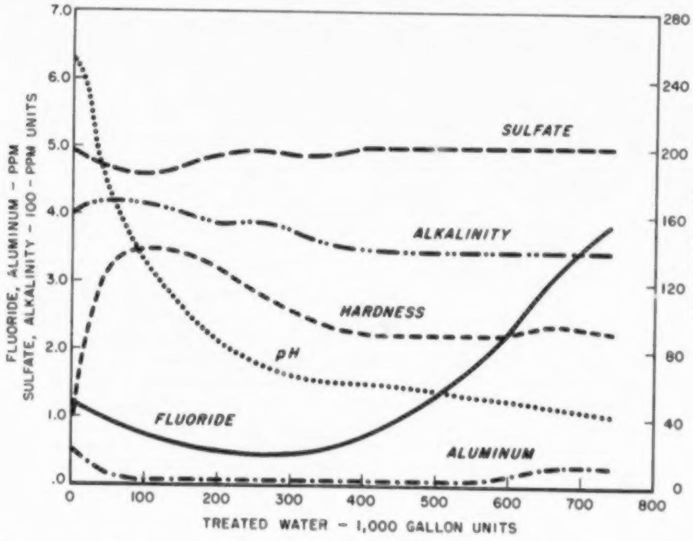

FIGURE 2.—*Fluoride level varies during treatment cycle at Bartlett, but average is 1 mg/L. A recent change as reflected in this graph, is to pretreat the raw water to pH 7.2.*

At present, the caustic solution is applied counter-currently which combines in one operation the backwashing step (which conserves the treated water formerly used for this purpose) and the caustic application. The caustic solution is applied at the rate of 235 gpm which, after passing the bed, is discarded. Dilution of this caustic in the sewer to the treatment plant (Hays Process) is sufficient to produce no adverse effect on sewage treatment or on the effluent from the treatment plant. About 28,000 gallons of raw water are then used for removing as much residual caustic as possible before the acid treatment. After refilling the caustic tank with spent rinse water the acid feeder is started and raw water at a reduced pH is used until the alkalinity approaches that of the raw water—requiring about 40,000 gallons. This amount of acid rinse also brings the starting fluoride level to 1.0 mg/L. The total amount of water used per regeneration amounts to about 68,500 gallons.

Recently a new well was drilled at Bartlett reaching 2,670 feet to the Trinity sands. This water is similar to the older well water but contains only 3.0 mg/L fluoride. One result of this change was that cycle lengths now extend to an average of 1.5 million gallons. However, because of the greater difficulty in removing fluorides at the lower concentrations, the capacity of the media fell to 400 grains of fluoride per cubic foot of media from the 700-grain level when using the 8.0 mg/L fluoride well. These removal capacities are based on reducing fluorides in the treated water to an equivalent of 1.0 mg/L and pretreating the raw water to reduce the pH to 7.2 with dilute sulfuric acid.

The equipment for the Bartlett plant cost $11,360 in 1951 including installation. The media cost an additional $4,000. The building, which includes space for a laboratory bench, connections to the sewer, to the wells and to the ground storage tanks and foundations for the tanks was furnished by the city. Chemical costs for the 3.0 mg/L well were $25/MG; for the 8.0mg/L well, $40/MG.

all costs FOB chemical manufacturers. Operation started on March 11, 1952.

USE OF BONE CHAR

The contact tank at Bartlett is open to the atmosphere and the water flows through the media by gravity. The plant at Britton, South Dakota, on the other hand, is a pressure type where the media is confined in a heavy steel tank designed to withstand the pressure of water maintained by elevated storage. The water at Britton is pumped from any of three wells through the contact tank at rates varying between 38 and 206 gpm and thence directly to the distribution system. This tank, equipped with a steel pipe-grid underdrain, cast iron caustic distributor and appropriate piping and valve manifold is 9 feet in diameter with a 10-foot shell height. It contains 300 cubic feet (56½ inches) of media resting on 12 inches of graded level. The media is 28-48 mesh bone char (manufactured primarily for use in sugar refineries for decolorizing sugar syrup) and at present the Britton installation is the only defluoridation plant in the United States using this material.

After treating about 450,000 gallons the media becomes saturated with fluoride and must be regenerated. This consists of backwashing with treated water (370 gpm for about 18 minutes) to remove the accumulated sand and then pumping 5,000 gallons of a 1 percent caustic soda solution through the bed. After the fluorides have been removed from the media by the caustic solution, the excess caustic remaining must be removed. This is done by first rinsing the bed with raw water (7,000 gallons at 150 gpm). After a point is reached where additional rinsing removes very little caustic, the bed is treated with a weak carbon dioxide solution. This solution is prepared by diffusing 84 pounds of carbon dioxide gas (from liquefied dry ice) through 8,500 gallons of raw water as it passes (at 150 gpm) a series of diffusers en route to the contact tank. When the pH of the waste water approaches that of the raw water the plant is ready for another cycle. There are 6,500 gallons of treated water used for backwashing together with 28,000 gallons of raw water used for regeneration.

Construction of this plant was completed and operation started November 20, 1948, with a synthetic hydroxy apatite for a media. This material was abandoned during February, 1953, because of excessive (42 percent per year) losses due to attrition. The cost of the equipment for the plant in 1947 was $12,245 including the media. Bone char of the type suitable for this purpose now sells for 4½ cents per pound, FOB manufacturer. A complete charge of 300 cubic feet costs $540. Current chemical costs (FOB manufacturer) for regeneration amount to $53 per million gallons.

MAGNESIUM PROCESS

No plants have yet been built specifically for fluoride removal using the magnesium process. However, there are several softening plants in States with hard-water wells such as Ohio, Indiana and Illinois that incidentally remove small amounts of fluoride along with the removal of magnesium. This combination of functions, using the same equipment and treatment chemicals would make the cost of defluoridation lower than any other process. On the other hand, the initial cost of building such plants is considerably higher and chemical and sludge handling is more expensive than for those plants using a chemical-regenerated media.

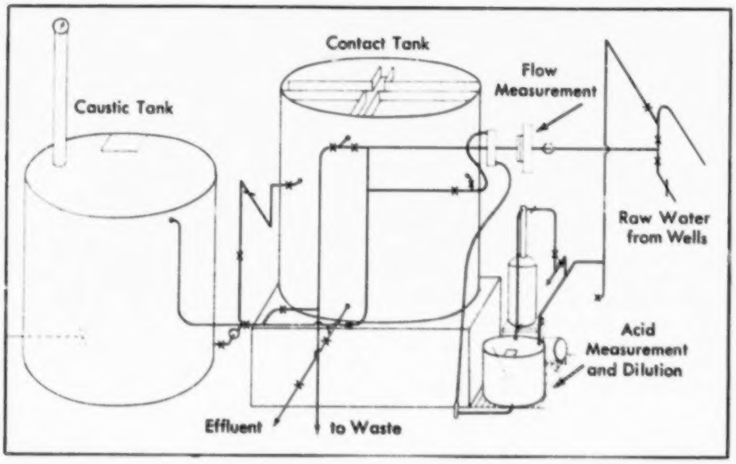

FIGURE 3.—*Diagrammatic layout of defluoridation unit at Bartlett. Contact tank holds activated alumina; regeneration is by caustic rinse and sulfuric acid neutralization.*

The magnesium process would probably be primarily used for water requiring softening and a simultaneous removal of fluorides from a level of 2.5 to 3.0 mg/L. A higher fluoride level using this process would probably require an excessive and uneconomical dose of lime. The choice between alumina and bone char cannot, at present, be based solely on a study of the chemical analyses of the untreated water. The decision as to which media or process should be used is now based on laboratory studies of the relative effectiveness and fluoride removal capacity of the two media with samples of the actual water to be treated.

The problems related to the operation, control and maintenance of defluoridation plants are no more difficult than those encountered in conventional water treatment plants. We have found that the employees of water systems previously involved only in well pump operation and meter reading could become capable defluoridation plant operators after adequate training. The equipment required for these defluoridation plants is a collection of standard water treatment plant items and their cost and complexity are identical to softening or ion exchange plants. Many plants have cited operating costs for softening, iron removal, decolorization, clarification or combinations of these processes which are similar to costs of defluoridation.

The removal of excess fluorides from community water supplies to prevent dental disfigurement, loss of teeth and increased cost of dental care is a sound public health procedure. The improved health of those using such water appears to justify defluoridation as much or more than other selected water treatment processes.

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Arnold, Francis A., Jr., D.D.S. Director. National Institute of Dental Research, 90. 101. 109. 123. 126. 150. 198. 200, 218, 223, 234. 253, 294, 302. 311. 402. 411. 471. 474
Bellack, Ervin, B.S. Chem. Eng., Division of Dental Public Health, 267. 579. 589. 593. 596, 601
Breaux, R. P., D.D.S. Division of Dental Public Health. 462, 466
Bullock, Frederick A., B.S. Laboratory of Biochemistry. National Institute of Dental Research. 549. 609
Carroll, James B., D.D.S., M.P.H. Dental Director, Fulton County Health Dept., Georgia. 244
Cohen, Chester Principal Assistant Engineer. Texas State Department of Health. 26
Chrietzberg, John E., D.D.S., M.P.H. Dental Director. Dept. of Public Health. Georgia, 244
Dean, H. Trendley, D.D.S. Former Director. National Institute of Dental Research, 11. 23. 26. 36. 41, 45. 49. 56. 62, 71. 85. 90. 101, 109. 123. 126. 198. 200. 218. 311. 462. 466
DeCamp, Floyd H., D.D.S. Director. Bureau of Dental Health, Florida State Board of Health. 183
Diffenbach, Viron, D.D.S. Regional Dental Consultant. 173
Dixon, R. M. District Sanitary Engineer. Texas, 26
Downs, Robert A., D.D.S., M.S.P.H. Colorado State Dental Health Director. 338
Eager, J. M., M.D. Supervising Surgeon-General. Marine Hospital Service, Naples. Italy. 2
Eastman, Paul W., Jr. Water Supply Consultant. 189
Elvove, Elias, Ph. D. Senior Chemist, National Institute of Dental Research. 36. 41. 49. 56. 62. 90. 101. 109. 123. 126. 166, 462. 466. 562. 619
Fath, Elizabeth H., B.S. Laboratory of Biochemistry. National Institute of Dental Research. 613. 616
Feldman, Floyd M. City Health Officer. Dept. of Health. Rochester. Minnesota. 320
Folk, John E., Ph. D. Biochemist. National Institute of Dental Research. 487
Galagan, Donald J., D.D.S., M.P.H. Chief. Division of Dental Public Health and Resources, 74, 206. 238. 322. 325
Geever, Ervin F., M.D. Pathology Consultant. Laboratory of Biochemistry. National Institute of Dental Research. 437. 452. 539
Geiser, Patricia, M.S. Clinical Investigations, National Institute of Dental Research. 402. 437. 452
Gillooly, Charles J., D.D.S. Regional Dental Consultant, 189
Hagan, Thomas L., D.D.S., M.P.H. Division of Dental Public Health, 432
Hamilton, Tom S., Ph. D. Professor of Animal Nutrition. University of Illinois, 377
Harris, W. L. Water Plant Supervisor. Dept. of Municipal Water and Light. Grand Rapids. Michigan. 274
Hasty, Frederick F., Jr., D.D.S. Regional Dental Consultant, 183
Hayes, Richard L., D.D.S., M.P.H. Epidemiology and Biometry Branch, National Institute of Dental Research. 223. 239
Heinz, Harold W., D.D.S. Director, Dental Health Services, Nebraska Dept. of Health. 189
Hilbish, Theodore F., M.D. Chief, Dept. of Diagnostic X-ray, National Institutes of Health. 414
Jay, Philip, D.D.S. Professor. University of Michigan School of Dentistry, 90, 101, 109. 198. 218
Jeffreys, Margaret H., M.P.H. Director. Division of Oral Hygiene. Delaware State Board of Health, 340
Johnston, David C., D.D.S. National Institute of Dental Research. 109. 126
Kempf, Grover A., M.D. Regional Consultant, 2
Kinser, Charles A., M.S. Chemist. National Institute of Dental Research, 370, 377
Klein, Henry, D.D.S., Sc. D. Dental Public Health Administrator. Division of Public Health Methods. 141. 144. 148. 155. 160
Knutson, John W., D.D.S., D.P.H. Former Chief Dental Officer, 198. 200. 213, 218, 306. 315, 317. 320. 322. 325. 328
Kornberg, Arthur, M.D. National Institute of Arthritis and Metabolic Diseases, 522
Kroschel, William P., D.D.S. Regional Dental Consultant. 183
Lamson, Glenn G., Jr., A.B. Health Program Representative, San Francisco. California, 74
Law, F. E., D.D.S., M.P.H. Chief. Disease Control Branch. Division of Dental Public Health and Resources. 340
Leatherwood, Ernest C., Jr., D.M.D., M.P.H. Epidemiology and Biometry Branch. National Institute of Dental Research. 186
Lee, Willard A., B.S. Laboratory of Biochemistry. National Institute of Dental Research, 435. 442, 457
Leone, Nicholas C., Ph. D. Chief. Medical Investigations. National Institute of Dental Research, 402. 411. 414. 435. 437, 442. 448, 452, 539
Lewis, Fred D., Jr., D.D.S. Dental Consultant. Georgia Dept. of Public Health, 186. 244
Lieberman, J. E., M.A. Mathematical Statistician, Office of Biometry. National Institutes of Health. 402. 437. 452
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Littleton, Norman W., D.D.S. Epidemiology and Biometry Branch, National Institute of Dental Research. 239
Loe, F. S., D.D.S. National Institutes of Health. 200
Maier, Franz J., B.S.M.E., B.S.C.E. Sanitary Engineer Director, Division of Dental Public Health. 258. 264. 267. 270. 299, 566. 579. 583. 589. 599. 622. 626, 631
McCann, Harold G., M.S. Chemist, Laboratory of Biochemistry. National Institute of Dental Research. 448. 546. 549, 604. 609. 613. 616
McCauley, H. Berton, D.D.S. National Institute of Dental Research. 200, 223. 394
McClure, Frank J., Ph. D. Chief. Laboratory of Biochemistry. National Institute of Dental Research. 90. 283. 287. 302, 346, 354. 361. 362. 370. 377. 384. 390. 392. 394. 421. 423. 442. 448. 457. 471, 474. 477. 480. 483. 487. 496. 505. 507. 521, 522. 523. 527. 533. 544. 552. 563
McKay, Frederick S., D.D.S. Consulting Specialist in Child Hygiene. Colorado. 2. 56. 71

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Mitchel, Harold H., Ph. D. Professor and Chief of Animal Nutrition, University of Illinois, 377, 496

Moran, Niel C., M.D. National Heart Institute, 539

Nevitt, George A., D.D.S. Regional Dental Consultant, 173

Pelton, Walter J., D.D.S. Chief, Manpower and Education Branch, Division of Dental Public Health and Resources, 338

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Poston, Richard F. Assistant Sanitary Engineer, South Dakota State Board of Health, 62

Presnell, Charles E., D.D.S. Dental Director, Missouri Division of Health, 173

Remsburg, Charles G. Chemist, National Institute of Dental Research, 562

Russell, A. L., D.D.S., M.P.H. Chief, Epidemiology and Biometry Branch, National Institute of Dental Research, 166, 200, 230, 245, 250, 253, 427

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Shimkin, Michael B., M.D. National Cancer Institute, 402

Shiota, T., Ph. D. Bacteriologist, National Institute of Dental Research, 491

Short, Edwin M., D.D.S. National Institute of Dental Research, 109, 126, 137, 200, 311

Singer, Leon, Ph. D. Department of Physiological Chemistry, University of Minnesota, 582

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